DETECTION OF MULTIDRUG RESISTANCE IN *KLEBSIELLA* SPECIES BY PHENOTYPIC AND GENOTYPIC METHODS IN A TERTIARY CARE HOSPITAL.

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BONAFIDE CERTIFICATE

This is to certify that this dissertation work entitled **"DETECTION OF MULTIDRUG RESISTANCE IN *KLEBSIELLA* SPECIES BY PHENOTYPIC AND GENOTYPIC METHODS IN A TERTIARY CARE HOSPITAL"** is the original bonafide work done by **DR.M.SATHIYA**, Post Graduate Student, Institute of Microbiology, Madras Medical College, Chennai under our direct supervision and guidance.

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DECLARATION

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Introduction

INTRODUCTION

Antimicrobial resistance (AMR) within a wide range of infectious agents is a public health threat of broad concern to countries [(1)]. Increasingly, governments around the world are beginning to pay attention to a problem so serious, that it threatens the achievements of modern medicine. AMR is a complex global public health challenge, and no single or simple strategy will suffice to fully contain the emergence and spread of infectious organisms that become resistant to the available antimicrobial drugs. The development of AMR is a natural phenomenon in microorganisms and is accelerated by the selective pressure exerted by use and misuse of antimicrobial agents in humans and animals[(2)]. The current lack of new antimicrobials on the horizon to replace those that become ineffective brings added urgency to the need to protect the efficacy of existing drugs.Hospitals, and particularly intensive care units, are an important breeding ground for the development and spread of antibiotic resistant bacteria. . An important cause of increasing antibiotic resistance is the selection of resistant bacterial strains by mutation and transfer of mobile resistance genes.Out breaks with a common source of multiple resistant bacteria ,often caused by organisms such as *Pseudomonas spp,Klebsiella spp,and Acinetobacter spp* are another hazard[(2,3)].

Klebsiella are ubiquitously present and reported worldwide[(4)]. In recent years *Klebsiella* have become important pathogens in nosocomial infections in the united states and india. *Klebsiella* are also important in nosocomial infections

among adult and pediatric populations. *Klebsiella* account for approximately 8% of all hospital-acquired infections , placing them among the top 8 pathogens in hospitals[5]. *Klebsiella* cause as many as 14% of cases of primary bacteremia, second only to *Escherichia coli* as a cause of gram-negative sepsis[6]. They may affect any body site, but respiratory infections and UTIs predominate. Mortality rates are as high as 50% and approach 100% in persons with alcoholism and bacteremia[7]. Epidemic and endemic nosocomial infections caused by *Klebsiella* species are leading causes of morbidity and mortality[8].

K.pneumoniae is a primary pathogen and can cause a classic form of primary pneumonia [2,9] *K.pneumoniae* can also cause urinary tract infection, nosocomial infections, wound and biliary tract infection, peritonitis, meningitis, bacteremia, enteritis, septicemia.[10]

K oxytoca is among the top 4 pathogens that cause infection in patients in neonatal intensive care units[11]. It is the second most frequent cause of gram-negative neonatal bacteremia. Pathogens that cause infection in patients in neonatal intensive care units. It is the second most frequent cause of gram-negative neonatal bacteremia.[11]

Extensive use of broad spectrum antibiotics in hospitalized patients has led to both increased carriage of *Klebsiella* and subsequently the development of multidrug resistant strains that produce extended spectrum betalactamase (ESBL,AmpC) and carbapenamase (KPC,MBL)[12]. These strains are highly virulent, show capsular type K55 and have extra ordinary ability to

spread(13).Most outbreaks are due to single clone or single gene.The bowel is the major site of colonization with infection of urinary tract,and wounds. Bacteremia and significant increased mortality have resulted from infection with these species[14].

In India it has been reported that 65.4%isolates were ESBL producers, 28.5were AmpCproducers, 9.4%were combined ESBL and AmpC producers and 48.6%were Carbapenamases producers in which 25.6%were KPC and 23% MBL producers and 8.2%were KPC and MBL coproducers [12,14,15]. For ESBL and AmpC producers, Carbapenems remain the drug of choice,where as in carbapenem resistant strains we are left with tigecycline and polymyxins which have started developing resistance to many GNBs.Hence the detection of Carbapenem resistance is important in treatment of patients and also preventing the spread of resistant strains[16].

The emergence and rapid spread of Multidrug resistant isolates of *Klebsiella* species causing nosocomial infections are of great concern worldwide[2,17]. Because of multidrug resistance of these isolates, it poses an intriguing problem to the treating clinician and increasing the mortality of the patients[18]. Hence invitro antimicrobial susceptibility pattern and identification of resistance pattern is important before treating *Klebsiella* infections.

Therefore the present study was undertaken to assess the most prevalent species among *Klebsiella* infections, the prevalent antibiotic sensitivity pattern, various resistance mechanisms among the isolates and the genes involved in Carbapenem resistance.This may provide the necessary information to formulate a hospital antibiotic policy and also to prevent the spread of multidrug resistance strains in the community.

Aims & Objectives

AIMS AND OBJECTIVES

AIMS:

To detect the Multidrug resistant Klebsiella species among various clinical specimens and to determine its resistance pattern by phenotypic and genotypic methods.

OBJECTIVES:

i) Isolation and identification of *Klebsiella spp* from various clinical specimens by standard microbiological procedures.

ii) Speciation of *Klebsiella* isolates obtained from various clinical specimens by standard microbiological procedures.

iii) Study of the antibiotic susceptibility pattern of the isolates as per CLSI 2016 Guidelines.

iv) To screen the presence of ESBL, AmpC betalactamases, Metallobetalactamases and Klebsiella pneumoniae carbapenemase among the *Klebsiella spp.*

v) To detect ESBL,AmpC,MBL and KPC with phenotypic confirmatory test among the *Klebsiella spp*.

vi) Detection of KPC bla gene in Multidrug resistant *Klebsiella spp* .

Review of Literature

REVIEW OF LITERATURE

Enterobacteriaceae

Enterobacteriaceae is a heterogeneous family of Gram-negative, nonsporulating, facultative anaerobic rods, belonging to the gamma-proteobacteria[2,20]. Most Enterobacteriaceae have type 1 pili (fimbriae), which enable bacterial adhesion to epithelial cells, and some species have flagella, which make them motile[4]. Essential biochemical characteristics of Enterobacteriaceae include fermentation of sugars (different species ferment specific sugars), reduction of nitrate to nitrite, oxidase negativityand catalase-positivity. Enterobacteriaceae thrive well at temperatures between 25°C and 37°C, and inhabit a wide spectrum of environmental, animal and human niches, such as the mammalian intestine, water, soil and plants [21].

The most clinically relevant Enterobacteriaceae may be divided into opportunistic pathogens, such as *Citrobacter* spp., *Enterobacter* spp. and *Klebsiella* spp., and overt pathogens, such as *Shigella* spp., *Salmonella* spp. and *Yersinia* spp.[4]. *E. coli* may be divided into commensal strains and pathogenic strains. *K. pneumonia* and *E. coli* are the most frequently observed Enterobacteriaceae in human clinical samples, and may cause common infections such as pneumonia, urinary tract infections (UTIs) and bloodstream infections (BSIs)[22].

Klebsiella species

According to Cowen's classification, *K. pneumoniae* may be divided into four subspecies: *K.pneumoniae,*subsp.*aerogenes, pneumoniae, ozaenae and rhinoscleromatis* [(23)]. The genus *Klebsiella* is named after the German-Swiss pathologist Edwin Klebs, who was the first to observe bacteria in the airways of patients who died of pneumonia in 1875. In 1882 the German pathologist and microbiologist Carl Friedländer detected bacteria in the fibrous exudate of patients who had died of lobar pneumonia, and it soon became apparent that two bacterial species could cause pneumonia, *Streptococcus pneumoniae* and *K. pneumoniae*, the latter also referred to as Friedländers bacillus [(24)].

Classification:

The Genus *Klebsiella* belongs to the tribe *Klebsiellae* , a member of the Family Enterobacteriaceae Facultative anaerobic,gram negative rods.[(25)]

FAMILY	:	Enterobacteriaceae
Species	:	*Klebsiella pneumonia*
		Klebsiella ornitholytica
		Klebsiella oxytoca
		Klebsiella planticola
		Klebsiella terrigena
Subspecies	:	*Klebsiella pneumoniae*
		Klebsiella aerogenes
		Klebsiella ozaenae

Klebsiella pneumoniae

Klebsiella rhinoscleromatis

K.planticola , *K.terrigena* and *K. ornithinolytica* are now transferred to a new genus *Raoultella* .

Cultural characteristics:

Members of the genus *Klebsiella* tend to be somewhat shorter and thicker than the other Enterobacteria and are straight rods about 1-2 µm long and 0.5-0.8 µm wide,with parallel or bulging sides and rounded or slightly pointed ends.The cells are either in pairs end to end or arranged singly.They are non-motile.when the capsule is pronounced ,it can be demonstrated even by Gram's stain[(26)].

Based on the presence or absence of capsular (K) somatic(O) and slime(M) antigens ,the Klebsiella strains have beem divided into 4 smooth and 4 rough forms[(27)].

	Smooth forms	**Rough forms**
1.	MKO	MKR mucoid capsulated
2.	KO	KR non mucoid capsulated
3.	MO	MR mucoid non capsulated
4.	O	R non mucoid non capsulated

When much capsular material is produced,the growth on agar is luxuriant,greyish white ,mucoid.and almost diffluent.This is due to high proportion of water-92%(Toenniessen1921)-in the capsular material[(28)].

The organisms are killed by moist heat at 55º in 30 min.They are survive drying for months (Loewenberg 1894)[29]. When kept at room temperature, cultures remain viable for weeks or months.They are Facultative anaerobic. There is no haemolysis of horse or sheep red cells.Lactose fermenting large moist glistening colonies due to polysaccharide capsule (K-antigen) in MacConkey agar.The optimum temperature for growth is 37º the limits are 12º and 43º[30].

Biochemical characteristics:

Biochemical characteristics used for the identification of *Klebsiella species* include: indole-test, production of lysine decarboxylase (but not ornithine decarboxylase), fermentation of specific sugars (e.g. D-glucose, lactose, sucrose, L-arabinose and maltose) and sugar-alcohols (e.g. D-mannitol). Furthermore, *Klebsiella* is non-motile and usually produce a prominent acidic polysaccharide based capsule[31]. Biochemical characteristics are still being used for species identification of bacteria isolated from clinical samples.

Species	indole	Gas	VP	Cit	Ure	Lac	Mal	Lys
Klebsiella pneumonia Subsp aerogenes	-	+	+	+	+	+	+	+
Klebsiella pneumonia Subsp pneumonia	-	+	-	+	+	+	+	+
Klebsiella pneumonia subsp ozaenae	-	*v*	-	*V*	-	*V*	-	*V*
Klebsiella pneumonia subsp rhinoscleromatis	-	-	-	-	-	+	-	-
Klebsiella oxytoca	+	+	+	+	+	+	+	+

Vitek 2 and phoenix are the two methods which are available for detection of Klebsiella from specimens,but their detection rate to identify the organism at the species level is poor.Hence it is not used routinely.

Pathogenicity and virulence factors:

The pathogenicity of *Klebsiella* spp. may be associated with virulence factors[32], such as

1) capsular antigens (O- and K-antigens),
2) adhesins,
3) siderophores
4) lipopolysaccharides (endotoxins).

The capsule is considered essential to the virulence of *Klebsiella*, as it protects the bacterium from phagocytosis and prevents killing of the bacteria by bactericidal serum factors[33]. Some serotypes or capsular types (K-types) of *K. pneumoniae*, e.g. K1, K2, K5,K54 and K57, have been associated with invasive human infectious diseases. K1 was observed among isolates causing Friedländer's pneumonia, and has more recently been associated with pyogenic liver abscesses[34,35]. Brisse *et al.* studied the association between K-type, sequence type (ST) and virulence gene content.

During recent years, several genes encoding virulence factors in *K. pneumoniae* have been described:

1) the plasmid-borne *rmpA*- regulates the mucoid phenotype [(36)],
2) *wcaG*- is associated with enhanced bacterial escape from phagocytosis
3) *kfu*- is involved in iron acquisition,
4) *fimH*- encodes type 1 fimbriae,
5) *mrkD*- encodes type 3 fimbriae and
6) *cf29A*- encodes the non-fimbrial adhesion factor CF29K [(38)].

K.pneumoniae ST23, which is frequently of serotype K1, is considered to be a particular virulent clone. Presence of *all S* is a marker for ST23 [(39)]. Calhau *et al*. Recently detected several virulence genes and pathogenicity islands (PAIs) in a collection of clinical ESBL-producing *K. pneumoniae* isolates from renal transplant patients [(40)]. In a recent Danish study, the virulence factors aerobactin, *kfu* and *rmpA* were detected in a hypermucoviscous *K. pneumoniae* ST23 blood isolate from a patient with a liver abscess [(41)].

Typing of Klebsiella isolates:

From an epidemiological point of view,it is often necessary to determine the clonality of the strains. This particularly important in endemic and epidemic nosocomial outbreaks of Klebsiella infections to improve the management of such out breaks [(42)]. A variety of methods have been used with various degrees of success in Klebsiella typing are mentioned as follows.

- Biotyping
- Serotyping
- Phage typing
- Bacteriocin (Klebocin) typing
- Molecular typing.

***Klebsiella spp* Intrinsic resistance:**

Klebsiella spp. are known to be inherent or intrinsically resistant to ampicillin ticarcillin and piperacillin due to chromosomal SHV-1-production. Furthermore, all Enterobacteriaceae are intrinsically resistant to penicillin G, glycopeptides, fusidic acid, macrolides (with some exceptions), lincosamides, streptogramins, daptomycin and linezolid[43]. Acquired resistance to other relevant antibiotic groups is increasingly reported in clinical *Klebsiella* isolates, and will be described more in, Biocides, including pesticides, preservatives for food, disinfectants and antiseptics, have been extensively used in agriculture, in food industry and in hospitals for decades. Bacterial tolerance to biocides has been observed, and concern has been raised on their impact on the selection of antimicrobial resistance (AMR) in human pathogens [44]. Efflux pumps extruding both multiple biocides and antibiotics have been described in Enterobacteriaceae and other Gram-negative bacteria [45]. Reduced susceptibility to the antiseptics chlorhexidine, trigene and benzalkonium chloride was recently documented in clinical *Klebsiella* isolates. The biocide resistance genes *cepA* and *qacE* (encoding efflux pumps) were detected in most of the isolates. There was, however, no genetic linkage between determinants encoding reduced biocide susceptibility and

antibiotic resistance [46]. Furthermore, bacterial resistance to heavy metals, such as copper and silver, also frequently used as antiseptics, has been reported in clinical MDR *Klebsiella* strains [47,48]. Altogether, the combination of antibiotic, biocide and heavy metal resistance in nosocomial pathogens, such as *Klebsiella species* may favour their selection and dissemination in the hospital environment.

Colonization in human and dissemination in hospitals

In humans, *Klebsiella species* may colonize the skin, the naso- and oropharynx and the intestinal tract [49,50]. Carriage rates of *Klebsiella species* are generally low in healthy humans, but have been found to increase dramatically in hospitalized patients, especially in patients treated with broad-spectrum antibiotics[51,52]. The healthy human skin is not a common reservoir for Gram-negative bacteria, but *Klebsiella species* has been found to colonize the hands of hospitalized patients and health care workers (HCWs) for several hours [53,54]. *Klebsiella* may spread remarkably well in the hospital environment, and frequently cause nosocomial infections and outbreaks, especially in intensive care units (ICUs). Medical equipment, the gastrointestinal tract of patients and the hands of HCWs are considered the most important reservoirs for the spread of *Klebsiella species* in the hospital environment [55,56].

***Klebsiella spp infections*:**

Historically, *K. pneumoniae* was known as the cause of community-acquired pneumonia or Friedländer's pneumonia, which particularly occurred in immuno compromised persons, such as chronic alcoholics. Friedländer's

pneumonia was a severe infection with high mortality if untreated[57]. This disease has become rare in most parts of the world. Today, *K. pneumoniae* is primarily known as a nosocomial pathogen, but may also be associated with community-acquired *K. pneumoniae* strains (often of serotype K1 and ST23) are increasingly observed in healthy subjects, especially in South East Asia[58]. Immuno-compromised hospitalized patients have an increased risk of opportunistic *K. pneumoniae* associated infections [59]. Furthermore, the rate of nosocomial *Klebsiella* infections has been found to be 4 times higher in patients who carry the bacteria in their intestine than in non-carriers [60].

K. pneumoniae belong to the top three or four pathogens causing ICU infections [61]. A combination of factors may explain why *Klebsiella species* infection is pneumonia or UTI, especially in elderly or immunocompromised persons. More lately,community-acquired liver abscesses with metastatic spread caused by highly virulent so common in the ICU setting. ,patient will have an suppressed immune system and lack a protective normal flora due to antibiotics[62,63]. Invasive procedures and ventilator support allow bacteria from the ICU environment to access the bloodstream and lungs [64]. Antibiotic treatment interferes with the development of the intestinal flora, and especially ampicillin has been associated with increased *Klebsiella* carriage rates in hospitalized in ICUs [65]. Lower UTIs caused by *Klebsiella species* may be successfully treated with per oral agents such as nitrofurantoin, trimethoprim and pivmecillinam. Agents available for treatment of upper UTIs and systemic *Klebsiella* infections include broadspectrum penicillins in combination with β-lactamase inhibitors (e.g.

piperacillin -tazobactam), fluoroquinolones, trimethoprim-sulfamethoxazole, aminoglycocides, broad-spectrum cephalosporins and carbapenems. However, resistance is emerging to most of these antibiotics, and the treatment options of *Klebsiella species* associated infections are becoming more and more limited[66].

Antibacterial agents:

The antibacterial effect of penicillin was discovered by Alexander Fleming in 1928[67]. Since the first antibacterial agents were taken into clinical use during the late 1930s and 40s, antibacterial chemotherapy has played a crucial role in the treatment of infectious diseases. Today's specialised modern medicine, like intensive care, cancer therapy and advanced surgery, rely on potent antibacterial agents. In the 1940s to 1960s, several antibiotic classes with different modes of action were detected and developed for clinical use. However, after the introduction of trimethoprim in 1968, no new classes of antibiotics effective in the treatment of Gram-negative bacterial infections have been discovered. The 3rd generation cephalosporins and a unique class of β-lactams, the carbapenems, were introduced in the 1980s. Furthermore, a large number of broad-spectrum cephalosporins and quinolones was introduced in the years to come, but these were all chemical modifications of agents already in clinical use [68]. The lack of new drugs effective in the treatment of Gram-negative bacterial infections along with the emergence of antibacterial resistance Gram-negative pathogens has become a serious threat for modern medicine.

Antibacterial agents differ in their mode of action, antimicrobial spectra, pharmacologic parameters (i.e. pharmacokinetics and -dynamics) and toxicity[69]. Antibacterial agents are frequently classified into five groups according to their mode of action and antibacterial target[70]:

(i) inhibitors of the cell wall synthesis (e.g. β-lactams, glycopeptides),

(ii) inhibitors of the DNA/RNA synthesis (e.g. quinolones,nitroimidazoles, rifampicin),

(iii) inhibitors of the folic acid synthesis (e.g.sulphonamides, trimethoprim),

(iv) inhibitors of the protein synthesis (e.g.aminoglycocides, macrolides, tetracyclines) and

(v) inhibitors of the cytoplasmic membrane (e.g. polymyxins) [71].

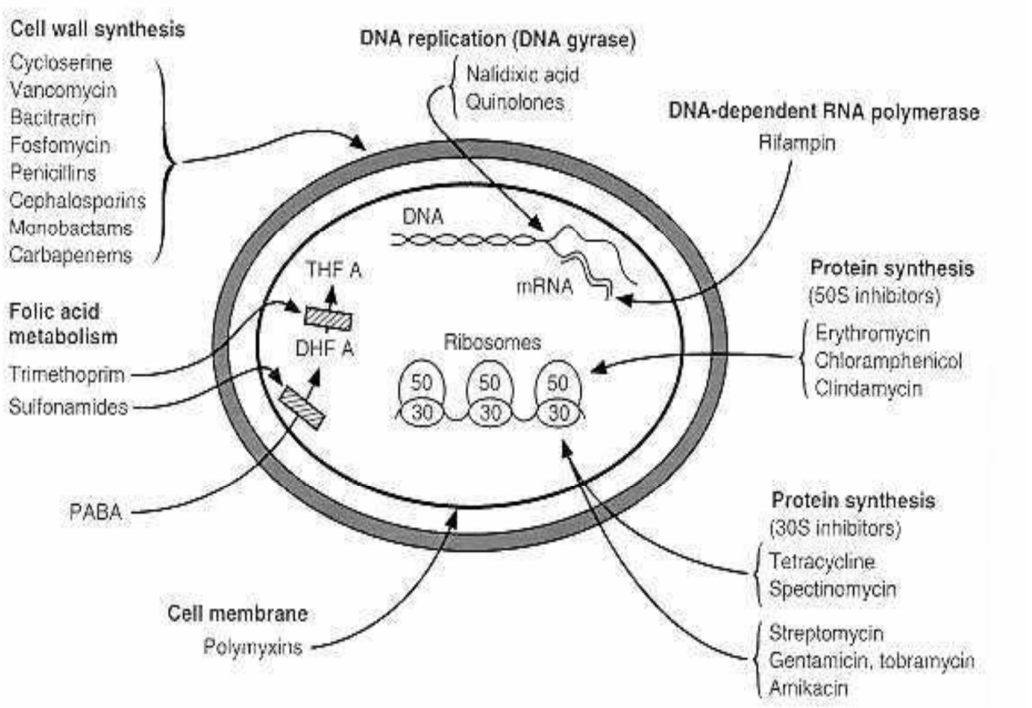


Fig. 1. Mechanism of drug resistance.

Definitions

Minimum ihibitory concentration:

The minimum inhibitory concentration(MIC) is the lowest antimicrobial concentration that inhibits visible growth of microorganism after overnight (ON) incubation *in vitro* [72]. MICs are used in microbiological laboratories to measure the activity of an antimicrobial agent against a specific microorganism.

Clinical susceptibility and resistance:

Since the 1970s, clinical breakpoints (based on MIC values) have been determined and used in microbiology laboratories to categorize microorganisms as susceptible (S), intermediate (I), or resistant (R). The S/I/R categorization provides guidance to clinicians with respect to the potential success of a given agent in the treatment of an infection, caused by a specific organism. S, I and R has been defined by the Clinical Laboratory Standard Institute on Antimicrobial Susceptibility Testing (CLSI) [11].

Microbiological resistance:

A microorganism is defined as wild type for a species by the absence of acquired resistance mechanisms to the drug in question. The epidemiological cut-off value (ECOFF) is a MIC value, which identifies the upper limit of measured MIC values for a given wild type population. ECOFFs are considered when setting clinical breakpoints, and they are useful indicators of developing resistance in surveillance studies. There is no systematic relationship between clinical breakpoints and ECOFFs [11].

Setting clinical breakpoints:

From a clinical point of view, AMR is a relative phenomenon influenced by the following factors, which must be considered when setting clinical breakpoints: available formulations of the agent (e.g. oral or intravenous), standard and maximum dosing, clinical indications, target organism, MIC distributions and ECOFFs for individual species (wild-type), pharmacokinetic (PK) and pharmacodynamic (PD) data, clinical data relating outcome to MIC values, information on resistance mechanisms and the clinical significance of the resistance mechanisms. Of notice, clinical breakpoints for susceptibility testing should not divide wild-type distributions of the target species [(11)].

Multi Drug Resistant(MDR):

The isolates resistant to atleast three classes of antimicrobial agents including all penicillins, cephalosporins, fluoroquinolones and aminoglycosides.

Extensive Drug Resistant(XDR):

The isolates will be resistant to carbapenems in addition to the MDR drugs.

Pan Drug Resistant(PDR):

The isolated will be resistant to all the available drugs,including polymyxins and tigecycline.

Antimicrobial susceptibility testing:

Antimicrobial susceptibility testing (AST) may be performed in several ways. The most common method used for AST in routine laboratories is

conventional disk diffusion, which categorize microorganisms as S, I or R. The use of plastic strips, containing an antimicrobial concentration gradient (gradient test), is a convenient way to generate MIC data on agar plates. Broth dilution is considered the gold standard of MIC determination, but is not commonly used in routine laboratories [11]. Recently, a standardized disk diffusion method (The CLSI method) was validated and implemented in several countries, including india [73,74]. Furthermore, automated AST systems (e.g. Vitek2 and Phoenix) are commonly used for AST in routine laboratories, and offer the convenience of combining species identification and MIC determination for relevant agents. introduced [75]. Due to widespread use of broad-spectrum cephalosporins, such as ceftazidime and cefotaxime, numerous TEM- and SHV-mutants with extended spectrum evolved.

β-lactams

Due to their diversity, broad spectrum of activity and low toxicity, β-lactams are the most prescribed antibiotics worldwide [76]. All -βlactams have the β-lactam ring in common. Due to differences in their side chains, β-lactams may be classified into the following main groups: penicillins, cephalosporins, monobactams and carbapenems (Table 1) [77]. β-lactams target the bacterial cell wall synthesis and act by binding covalently to penicillin binding proteins (PBPs). PBPs are bacterial enzymes involved in the synthesis and crosslinking of peptidoglycan, which is a major component of the bacterial cell wall. PBPs are located in the inner cytoplasmic membrane or in the periplasmatic space of Gram-negative bacteria. When PBPs are inactivated by β-lactams, the peptidoglycan

synthesis is inhibited and the bacterial growth is affected. Irregularities in the cell wall synthesis lead to loss of integrity and finally cell lysis [78].

Resistance to antibacterial agents

Since antibacterial agents were taken into clinical use, resistance to most classes of relevant antibiotics, including the β-lactams, has emerged among human pathogens. Sir Alexander Fleming warned in his Nobel lecture in 1945 that resistance may evolve if antibacterial drugs are misused or under-dosed: "It is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them, and the same thing occasionally happened in the body". (Alexander Fleming, Nobel lecture "Penicillin", 1945)[79]. Resistance to antimicrobials is a natural biological phenomenon. The introduction of every antimicrobial agent into clinical practice has been followed by the detection in the laboratory of strains of microorganisms that are resistant, i.e. able to multiply in the presence of drug concentrations higher than the concentrations in humans receiving therapeutic doses. Such resistance may either be a characteristic associated with the entire species or emerge in strains of a normally susceptible species through mutation or gene transfer. Resistance genes encode various mechanisms which allow microorganisms to resist the inhibitory effects of specific antimicrobials. These mechanisms offer resistance to other antimicrobials of the same class and sometimes to several different antimicrobial classes (WHO). Gram negative bacteria use four mechanisms of resistance to survive to the antibiotic treatment [80].

Efflux of antibiotics from bacteria

Efflux pumps play a major role in antibiotic resistance and also serve other functions in bacteria such as the uptake of essential nutrients and ions, excretion of metabolic end products and deleterious substances as well as the communication between cells and environment [(81)].

Outer membrane (OM) permeability:

The OM of Gram negative bacteria is a barrier to both hydrophobic and Hydrophilic compounds. By combining a highly hydrophobic lipid bilayer with pore forming proteins of specific size-exclusion properties, the OM acts as a selective barrier. The permeability properties of this barrier have a major impact on the susceptibility of the microorganism to antibiotics, which are essentially targeted at intracellular processes[(82)]. Small hydrophilic antibiotics, such as, β-lactams, use the pore forming proteins (water filled channel proteins embedded in the outer membrane, e.g., OmpF in *E. coli* and OprD in *Pseudomonas aeroginosa*) to gain access to the cell interior, while macrolides and other hydrophobic antibiotics diffuse across the lipid bilayer. The existance of antibiotic-resistant strains in a large number of bacterial species due to modifications in the lipid or protein composition of the OM indeed highlights the importance of the OM barrier in antibiotic sensitivity [(83)].

Target modifications

This mechanism is based on alterations of bacterial sites that are targeted by antibiotics and thus preventing the antibiotic from binding to its site of action.

For example fluoroquinolone resistance is attributed to mutations within the drug.s target (DNA gyrase and topoisomerase) [84].

Enzymatic modification of the antibiotic:

Enzymes that modify antibacterial antibiotics are devided into two general classes: a) βlactamase that degrade antibiotics and b) others (including the macrolide and aminoglycoside-modifying proteins) that perform chemical transformations to render the antibiotic inefficient [84].

The acquisition and spread of antibiotic resistance in bacteria:

Multiple drug resistant strains of some bacteria have reached the proportion that virtually no antibiotics are available for treatment[85]. Antibiotic resistance in bacteria may be an inherent trait of the organism (e.g. a particular type of cell wall structure) that renders it naturally resistant, or it may be acquired by means of mutation in its own DNA or acquisition of resistance-conferring DNA from another source [86].

Inherent (natural) resistance:

Bacteria may be inherently resistant to an antibiotic. For example, an organism lacks a transport system for an antibiotic; or an organism lacks the target of the antibiotic molecule; or, as in the case of Gram-negative bacteria, the cell wall is covered with an outer membrane that establishes a permeability barrier against the antibiotic[87].

Acquired resistance:

Several mechanisms are developed by bacteria in order to acquire resistance to antibiotics. All require either the modification of existing genetic material or the acquisition of new genetic material from another source[87].

Vertical gene transfer :

The spontaneous mutation frequency for antibiotic resistance is on the order of about of about 10-8- 10-9. Once the resistance genes have developed, they are transferred directly to all the bacteria's progeny during DNA replication. This is known as vertical gene transfer or vertical evolution[88].

Horizontal gene transfer

Lateral or horizontal gene transfer (HGT) is a process where genetic material contained in small packets of DNA can be transferred between individual bacteria of the same species or even between different species. There are at least three possible mechanisms of HGT, equivalent to the three processes of genetic exchange in bacteria. These are transduction, transformation or conjugation[89].

Conjugation

Occurs when there is direct cell-cell contact between two bacteria (which need not be closely related) and transfer of small pieces of DNA called plasmids takes place. This is thought to be the main mechanism of HGT.

Transformation

It is a process where parts of DNA are taken up by the bacteria from the external environment. This DNA is normally present in the external environment due to the death and lysis of another bacterium.

Transduction

Occurs when bacteria-specific viruses (bacteriophages) transfer DNA between two closely related bacteria [89].

Mechanisms of resistance

Antibacterial resistance may be intrinsic (natural) or acquired. Intrinsic bacterial resistance to antibacterials, produced by other bacteria or fungi, existed in the environment before antibacterial compounds were taken into clinical use. Bacteria, furthermore, have the remarkable ability of environmental adaptation by changing their genome through mutations or by horizontal gene transfer (HGT), or by differential gene expression [46, 90]. The extensive use of antibiotics in humans, but also in food animals and fish farming, has led to a selective pressure in several environmental niches promoting acquisition of resistance determinants.

The mechanism of resistance in Klebsiella involves the following three broad categories[7]

1) Antimicrobial inactivating enzymes.
2) Reduced access to bacterial targets.
3) 3)Point mutations that change targets or cellular functions.

Resistance may be acquired in three ways[91]

(i) mutations in chromosomal genes,causing altered antibacterial targets or transcriptional changes, e.g. mutation inchromosomal topoisomerase / gyrase genes leading to fluoroquinolone resistance ,

(ii) acquisition of new genes by horizontal gene transfer (HGT) , e.g. plasmid mediated acquisition of β-lactamase encoding genes , or

(iii) mutations in previously acquired genes, e.g. mutation in the β-lactamase genes *bla*TEM-1 and *bla*SHV-1, resulting in production of enzymes with a broader spectrum .

Four biochemical mechanisms of antibacterial resistance have been described[92]:

(i) inactivation or modification of antibiotics by antibiotic-modifying enzymes,e.g. β-lactamases and aminoglycoside modifying enzymes ,

(ii) modification of the target molecule.

(iii) restricted access to the target of an antibiotic due to reduction of porins in the outer membrane of Gram-negative bacteria, and (iv) efflux of one or more antibiotic groups from the bacterial cell due to efflux pumps in the cytoplasmic membrane. Bacteria may combine two or more of these mechanisms.

Mechanisms of β-lactam resistance:

Resistance to β-lactams may be PBP-mediated, or caused by production of β-lactamases. Porin loss or down-regulation may cause β-lactam resistance alone, or in combination with β-lactamase production. PBP-mediated resistance may be

caused by acquisition of foreign PBPs, e.g. acquisition of the gene encoding PBP2a in methicillin resistant *S. aureus* (MRSA) [57], or by modifications of PBPs, e.g. penicillin non-susceptible *S. pneumoniae* (PNSP) due to mosaic PBPs[57].

β-lactamase production is the most common mechanism of β-lactam resistance Gram-negative bacteria. β-lactamases are enzymes, which may inactivate β-lactam antibiotics by hydrolysing the amide bond of the β-lactam ring[93]. β-lactamases may be classified based on their primary structure according to Ambler [91], or due to their functional characteristics (i.e. the enzymes abilities to hydrolyse different β-lactam classes) according to Bush-Jacoby-Medeiros [91-94]. The serine β-lactamases (Ambler class A) share several highly conserved amino acid sequences with PBPs, from which they probably evolved [95,96].

As for the β-lactam antibiotics, also the β-lactamases vary in their spectrum of activity depending on the structure of their side chains, and they may be subdivided into[91]:

(i) narrow-spectrum β-lactamases (penicillinases),

(ii) broad-spectrum β-lactamases (ampicillinases), (iii) extended-spectrum β-lactamases (ESBLs), which may hydrolyse 3rd and 4th generation cephalosporins and monobactams, and (iv) carbapenemases, which may hydrolyse all β-lactams, including the carbapenems.

β-lactams and β-lactamases in a historical perspective:

As penicillin was taken into clinical use during the early 1940s, it was considered the "magic bullet" against all Gram-positive bacteria [(91)]. In 1983, the first *K. pneumoniae* isolate resistant to 3rd generation cephalosporins was discovered in Germany [(92)]. The new β-lactamase, which conferred resistance to cefotaxime, was a mutation-driven alteration of the existing SHV-1 enzyme, and thus designated SHV-2 [(93)]. The first TEM-derived β lactamase conferring resistance to cefotaxime, TEM-3 (initially named CTX-1), was reported in *K. pneumoniae* from France a few years later [(74, 75)]. In 1988, the term extended-spectrum β-lactamase (ESBL) was introduced [(76)]. Due to widespread use of broad-spectrum cephalosporins, such as ceftazidime and cefotaxime, numerous TEM- and SHV-mutants with extended spectrum evolved.

During the early 1990s, ESBL-producing *K. pneumoniae* emerged as a nosocomial pathogen, causing hospital acquired infections and hospital outbreaks[(92-93)]. In 1989, a clinical cefotaxime resistant *E. coli* isolate, producing a non-TEM/SHV ESBL, was recognized in Munich. The new enzyme was designated CTX-M due to its predominant activity against cefotaxime rather than ceftazidime, and the geographical reference [(94)]. A shift from the predominance of TEM- and SHV-enzymes among *K. pneumoniae* in the hospital setting to the dissemination of CTX-M-enzymes among *E. coli* and *K. pneumoniae*, also in the community, was observed [(94)].

The beta-lactamases are classified by two systems:

Ambler's classification : it is a molecular classification based on aminoacid sequences.

Bush-jacoby Medeiros classification:it is functional classification.

Ambler's classification:

Class A : Pencillinase (eg.TEM,SHV)

Class B : Metallobetalactamase (eg.IMP,VIM)

Class C : Cephalosporinase-AmpC (eg.CMY,NMC)

Class D : Oxacillinase (eg.OXA 23,OXA 58)

Class A,C,D : require serine moieties for their function,similarly

Class B : require Zinc for its action.

Bush-jacoby Medeiros classification:

Group	Enzyme	Molecular class	Inhibited by Clavulanicacid.
1	Cepalosporinase	C	No
2a	Pencillinase	A	Yes
2b	Broad spectrum	A	Yes
2be	Extended spectrum	A	Yes
2br	Inhibitor resistant	A	Diminshed
2c	Carbenillinase	A	Yes
2d	Cloxacillinase	D or A	Yes
2e	Cephalosporinase	A	Yes
2f	Carbapenamase	A	Yes
3	Carbapenamase	B	No
4	Penicillnase		No

AmpC Betalactamases:

The first plasmid-mediated AmpC β-lactamases were reported in *K.pneumoniae* in 1990 [95]. AmpC type Cepalosporinase are inherently expressed by Klebsiella pneumoniae.These will hydrolase aminopencillins and extended spectrum cephalosporins.The carbapenem resistance is also mediated by AmpC beta lactamases when present along with decreased membrane permeability or due to alterations in pencillin binding proteins.

Extended spectrum beta lactamases(ESBLs):

Ambler class A beta lactamases are reported in Klebsiella species.The first ESBL reported in Klebsiella pneumonia was TEM-3 in France initially,but now it has spread throughout the world.It is either chromosomal or plasmid mediated requires insertion sequence ISPa 12 for its expression.The other ESBLs reported in Klebsiella pneumonia are TEM-1,TEM-2,CTX-M2,CTX M43,PER-1,VEB-1also been reported.

Coproduction of ESBL and AmpC Betalactamases:

The genus Klebsiella has high level expression of the natural production of AmpC type betalactamases.The AmpC producing organism can act as hidden reservoir for the ESBLs.The high level expression of AmpC βlactamases may mask the recognition of the ESBLs and it may result in fatal and inappropriate antimicrobial therapy[96]. Hence the detection of ESBL among AmpC producers helps in the appropriate treatment of the patients.The coproduction of ESBL and AmpC in Klebsiella was reported as 15.4%.

Carbapenamases:

Carbapenems are broad spectrum antibiotics structurally very similar to penicillin but contain a sulphur group at C1 position. Several Gram-negative pathogens have become resistant to these by acquiring any one or more of the following mechanisms: structural alterations in drug targets like penicillin binding proteins (PBPs), porin loss, upregulation of efflux pumps and expression of carbapenemases[1]. One of the most commonly observed mechanism among Gram-negative pathogens is production of carbapenem inactivating enzymes, called carbapenemases[(88)]. All carbapenemases are β-lactamases but not all β-lactamases are carbapenemases. These periplasmic enzymes hydrolyze beta lactam antibiotics either by alteration in the target site of the antibiotic that reduces its binding capacity or modification of the antibiotic so that it is no longer recognized by the target. Carbapenemases are broadly divided into two major types based on the amino acid sequences: metallo β-lactamases (Class B) containing zinc at the active site whereas serine β-lactamases (Classes A, C and D) containing serine at the active site. The *Klebsiella pneumonia* carbapenemases (KPC) along with other members like SME, IMI, NMC, GES constitute the class A. Among these, the carbapenemase is more important than others due to its prevalence and transmissibility through plasmids. Class A carbapenemase production has become a most common mechanism of resistance across the world[(96)]. The KPCs are encoded on a conjugative plasmid, carried in Tn*3* (Tn*4401*) based transposon[(97)]. Though class A enzymes and Bush's 2f type of hydrolases[21] are comparable in many characteristics, the KPC enzymes have two unique features: *(i)* these are

borne on transferable conjugative plasmids, and *(ii)* capable of hydrolyzing advanced derivatives of cephalosporins like cefotaxime, Tazobactam and clavulanic acid are partial inhibitors of class A enzymes whereas boronic acid acts as complete inhibitor. Thirteen variants of KPC ($bla_{\text{KPC 1-13}}$) have been identified till date, which differ from each other by non-synonymous mutation[97].

Since 1990 several new ESBL-groups conferring resistance to the carbapenems have been detected in clinical isolates all over the world. Imipenem resistance in *P. aeruginosa* due to a plasmid-mediated metallo-β-lactamase (MBL) was reported from Japan in 1991 [98]. The first carbapenem hydrolysing OXA-enzyme (OXA-23) was described in *Acinetobacter baumanii* in 1993. OXA-48 was identified in a Turkish carbapenem resistant *K. pneumoniae* isolate in 2001 . The first *K. pneumonia* carbapenemase (KPC-1) was reported from USA in 2001 [98]. In 2009, a carbapenem resistant *K. pneumoniae* isolate, producing a novel MBL, designated New-Delhi metalloβlactamase-1 (NDM-1), was isolated from a Swedish patient. NDM-1 has the potential to spread rapidly among clinically relevant Enterobacteriacea . Thus, the detection of this enzyme attracted much attention and publicity. The current spread of the carbapenamases KPC, Metallobetalactamases (MBLs) like VIM, IMP and NDM among Enterobacteriaceae cause great concern [98]. MBL genes are mobilegenetic elements that can be transferred easily which pose a great threat of spread.[45]

KPC (*K. pneumoniae* carbapenemase) (Class A)

A few class A enzymes, most noted the plasmid-mediated KPC enzymes, are effective carbapenemases as well. Three variants are known, distinguished by one or two amino-acid substitutions. KPC-1 was found in North Carolina, KPC-2 in Baltimore and KPC-3 in New York. Plasmid .borne KPC enzymes are emerging among *K.pneumoniae* and other *Enterobactericeae*[13]. The class A *Klebsiella pneumonia* carbapenemase (KPC) is currently the most common carbapenemase, which was first detected in North Carolina, USA, in 1996 and has since spread worldwide.A later publication indicated that Enterobacteriaceae that produce KPC were becoming common in the United States [45].

NDM-1 (New Delhi metallo-β-lactamase)

Originally described from New Delhi in 2009, this gene is now widespread in Escherichia coli and Klebsiella pneumoniae from India and Pakistan. As of mid-2010, NDM-1 carrying bacteria have been introduced to other countries (including the USA and UK), presumably by medical tourists undergoing surgery in India. Inhibitor-Resistant β-Lactamases (Wikipedia 2010).

Resistance to Quinolones:

Fluoroquinolone resistance is mediated by DNA topoisomerase,acquisition of mobile genetic elements or through efflux pumps. The mutation in topoisomerase enzyme like gyrA and parC leads to the modification of lipopolysaccharides which also confers resistance in K.pneumoniae.[36,49]

Resistance to Aminoglycosides:

Plasmid or transposons coded Aminoglycoside - Modifying Enzymes (AMEs) or Efflux pumps are involved in aminoglycoside resistance.[35]

Resistance to Tigecycline:

The over expression of AdeABC multidrug efflux pump confers resistance to Tigecycline and also to many other antibiotics like tetracycline, aminoglycosides and quinolones.[97]

Resistance to Colistin:

The modification in the Lipopolysaccharides of the bacterial cell membrane due to point mutation interfere with the binding of the antimicrobial agents like Colistin.[98]

LABORATORY METHODS TO DETECT RESISTANCE MECHANISMS:

Phenotypic Screening methods:

Extended Spectrum Betalactamase Detection:

ESBLs are capable of hydrolyzing penicillins-oxyiminocephalosporins and Monobactams (Aztreonam) and are inhibited by Betalactamase inhibitors but have no detectable activity against Cephamycins or Carbapenems.Isolates exhibiting resistance to one or more 3rd generation cephalosporinslikecefotaxime (30µg/ml≤22mm), Ceftrioxone (30µg/ml≤19mm), Ceftazidime (30µg/ml≤17mm) with reference to CLSI 2016 AST interpretive criteria are considered to be ESBL producers.[45]

AmpC betalactamase Detection:

AmpC betalactamases are resistant to betalactamase inhibitors,all betalactams including Cephamycins except Carbapenems.Isolates showing resistance to Cefoxitin(30µg/ml≤14mm),should be considered as probable AmpC producers.[60,63,64]

Carbapenemase Detection:

Carbapenamases are capable of hydrolyzing carbapenems,other betalactams and betalactamase inhibitors with the exception of Aztreonam.With reference to CLSI 2016 document,disc diffusion testing using discs with 10µg potency of Imipenem and Ertapenem is used as a screening test for carbapenamase production.The isolates having zone diameter ≤19mm and ≤18mm with imipenem and Ertapenem respectively are categorized as resistant.[48]

Confirmatory methods:

Extended Spectrum Betalactamase:

CLSI phenotypic confirmatory method.

Double disc diffusion synergy test.

Three dimensional test

Modified Three dimensional test

Inhibitor potentiated disc diffusion test

ESBL E strip method.

Automated methods.

AmpC Betalactamases:

Modified Three dimensional test

AmpC disc test

Detection by Cefoxitin agar media

Detection by inhibitor based method

AmpC Betalactamase E test.

Carbapenamase Detection:

Imipenem-EDTA combined disc test.

Imipenem-EDTA double disc diffusion test.

Ertapenem-Phenylboronic combined disc test.

Modified Hodge test.

MBL E test.

KPC E test.

Molecular methods:

Polymerase Chain Reaction (PCR) is a technique which amplifies a specific DNA target ,so as to obtain a million or more copies which can then be easily visualized by using DNA staining techniques for the identification of resistance conferring genes.PCR is the gold standard procedure to determine the resistant genes,but cost prohibiting.[64].

Therapeutic options:

The therapeutic options for the management of MDR,XDR,and PDRof Klebsiella spp.infections have declined due to emergence and disseminationof antimicrobial resistance even to many last line drugs.

Treatment of MDR Klebsiella species:

Carbapenem:

Carbapenem remains the drug of choice for the treatment of MDR Klebsiella species.MYSTIC surveillance program documented that Imipenem is more potent than Ertapenem for because efflux pumps affect Ertapenem to a greater degree when compared to imipenem.Hence Imipenem and Ertapenem susceptibility should be done[(99)].

Beta lactam /Beta lactamases inhibitor:

As ESBL producing Enterobacteriaceae are frequently susceptible in vitro to Beta lactam/ Beta lactamase inhibitor combinations, it is logical to assume these combinations would also be clinically effective .But here we have to know that AmpC enzymes are normally resists.

Quinolones

If there is in vitro susceptible to ciprofloxacin, a satisfactory clinical response can be achieved by using quinolones [(66)].

Aminoglycosides

As in the case with quinolones, aminoglycosides are effective therapy against ESBL producing pathogens. Susceptibility to amikacin seems to be preserved, in contrast to gentamicin and tobramycin, thus justifying its use as empiric therapy [(84)].

Treatment of XDR and PDR Klebsiella species:

Few antimicrobials are currently available to treat infections with carbapenemase-producing Gram-negative bacteria. Carriage of concurrent resistance determinants can result in decreased susceptibility non-β-lactams including the fluoroquinolones and aminoglycosides thus further compromising an already limited antimicrobial arsenal. What frequently remains available are the polymyxins (including colistin), tigecycline, and fosfomycin but susceptibilities to these agents are unpredictable[(88)].

polymyxin B:

The reintroduction of polymyxins, both polymyxin B and colistin overlaps with the evolution of carbapenem resistance among Gram-negative bacilli.

Tigecycline

Tigecycline, a novel, first in class glycycline and an analogue of the semisynthetic antibiotic minocycline, is a potent, broad spectrum antibiotic that acts by inhibition of protein translation in bacteria by binding to the 30S ribosomal subunit and blocking the entry of amino-acyl to RNA molecules into the A site of the ribosome [(34)].CLSI criteria to interpret susceptibility testing of

tigecycline are not yet established. The guideline laid down by FDA was used in many studies. *In vitro* data supports the notion that tigecycline can be considered an alternative to carbapenems for treatment of infections due to ESBL-producing *Enterobacteriaceae*.High degree of resistanceto tigecycline has also been reported in Klebsiella species.

Fosfomycin

Fosfomycin tromethamine is a soluble salt of fosfomycin with improved bioavailability over fosfomycin. It inactivates the enzyme pyruvyltransferase, which is required for the synthesis of the bacterial cell wall peptidoglycan [(34)]. The excellent *in vitro* activity of fosfomycin against ESBL-producing *E. coli* and *K. pneumoniae* strains has been recently reported. Further studies are required to assess the efficacy of fosfomycin for the treatment of UTIs caused by ESBLproducing enterobacteria [(52)].

Colistin

Although once considered a toxic antibiotic, clinicians have now turned to colistin as a last resort agent for the treatment of infections caused by multidrug resistant gram negative bacteria, against which this cationic detergent-like compound remains active. The antimicrobial target of colistin is the bacterial cell membrane, where the polycationic peptide ring interacts with the lipid A of Lipopolysacharides, allowing penetration through the outer membrane by displacing Ca+ and Mg+ . Insertion between the phospholipids of the cytoplasomic membrane leads to loss of membrane integrity and to bacterial cell

death [34]. As mentioned above, carbapenem resistance mediated by KPC is emerging among enterobacteria. Their implication in outbreaks, as seen in hospitals in New York City, has created a context in which the empiric use of colistin is necessary. The cephamycins, including cefoxitin and cefotetan, are stable to hydrolysis by ESBL producing Enterobactericeae. However, there is a general reluctance to use these agents because of the relative ease by which some isolates may decrease the expression of outer membrane proteins, thus creating resistance to these agents during therapy.

Control measures:

Proper infection control practices and barriers areessential to prevent spread and outbreaks of ESBL producing bacteria. The reservoir for these bacteria seems to be gastrointestinal tract, oropharynx, colonized wounds and urine. The colonized hands, equipment could help in spreading infection between patients. So, mandatory infection control practices would be hand washing, barrier precautions, isolation of colonized/infectedpatients. Surveillance of patients of ICUs will help inearly detection and control practices related to ESBL and Carbapenamases production. Antibiotic restriction and antibiotic cycling especially the empirical use of higher generationcephalosporins and carbepenems are other measureswhich if monitored properly, could help in control of the emergence and *Multi drug resistant Klebsiella spp*.

Infection control practices:

1. Standard precautions,environmental cleaning,and disinfection.
2. Point source control effective during outbreak.
3. Contact barrier precaution to health care personnel.
4. Cohorting of patients.
5. Cohorting of health care personnel.
6. Clinical unit closure during outbreak to interrupt transmission and for thorough environmental disinfection.
7. Judicious use of antimicrobials to prevent drug resistance by antimicrobial stewardship.
8. Passive and active surveillance to identify colonized or infected patients, so that interventions can be implemented.

Materials & Methods

MATERIALS AND METHODS

Place of study:

This cross sectional study was conducted in the Institute of Microbiology, Madras Medical College, Rajiv Gandhi Government General Hospital, Chennai-3.

Study period:

The study period was for one year from September 2016 to August 2017.

Ethical consideration:

Approval was obtained from the Institutional ethics committee before the commencement of the study. Informed consent was obtained from all the patients who participated in this study. All the patients satisfying the inclusion criteria were included. Patients were interviewed by structured questionnaire.

Statistical analysis:

Statistical analyses were carried out using Statistical Packages for Social Sciences (SPSS). The proportional data of this cross sectional study were using Pearson's Chi Square analysis test & Fisher Exact test.

Study Population:

A total of 200 clinically significant, consecutive, non-duplicate isolates of *Klebsiella* spp. were included in this study. The isolates were from various clinical specimens sent to the Institute of Microbiology for bacteriological culture, biochemical identification and antibiotic susceptibility testing. Isolates included in this study were obtained from blood, sputum, endotracheal aspirate , bronchial

wash, pleural fluid, ascitic fluid, peritoneal dialysis fluid, cerebrospinal fluid, urine and wound swabs.

Inclusion criteria:

1. Clinically significant, consecutive, non-duplicate isolates were included in the study. The significance of the isolate was based on two or more of the following criteria-clinical history, presence of organism in Gram stain, presence of intracellular forms of the organism and pure growth in cuture with a significant colony count wherever applicable.
2. Patients aged more than 18 years.

Exclusion criteria:

1. Isolates of repeated samples from the same patient were not included in the study.
2. Patients aged less than 18 years were not included.

Preliminary identification of isolates belonging to genus *Klebisella* was done based on the following characteristics.

4.1 COLONY MORPHOLOGY:

- **On Nutrient agar** – large sized greyish white with smooth surface, mucoid and opaque colonies without any pigmentation without any specific odour.
- **On Blood agar** – mucoid circular colonies without hemolysis.
- **On MacConkey agar –**mucoid lactose fermenting colonies.
- **On CLED –** circular, 1-2 mm in diameter mucoid lactose fermenting colonies.

- The isolates obtained were subjected to preliminary tests like Gram staining, Catalase test , Oxidase test and Motility by Hanging drop method**.**
- The isolates which were Gram negative bacilli, catalase positive ,oxidase negative and non – motile by hanging drop were subjected to biochemical reactions for further confirmation.
- The following preliminary biochemical reactions were done with appropriate controls – Triple sugar iron agar medium for sugar fermentation and hydrogen sulphide production, Indole production using Kovac's reagent and Citrate utilization on Simmons Citrate Medium. Urease production test on Christensen's urease medium. Methyl red test for acid production and Voges proskauer test for acetoin production, lysine decarboxylation .
- Isolates giving the following reactions were further processed in the study:- by Moller's decarboxylase medium.

- **Triple Sugar iron agar –** Acid slant /acid butt with abundant gas,no hydrogen sulphide production.
- **Indole** was not formed on adding Kovac's reagent to 24hr broth culture, formed only in *Klebsiella oxytoca* isolates .
- **Presence** of growth or change in colour from apple green to blue denotes utilization of citrate.

SPECIATION OF *KLEBSIELLA* ISOLATES

Phenotypic characterization:

The isolates which were identified as belonging to the Genus *Klebsiella* were subjected to the following biochemical reactions for speciation.

- **Hugh &Leifson's OF medium:** A set of semisolid medium containing 1% glucose was inoculated with a young agar slope culture. One of the tube was immediately overlaid with sterile paraffin oil to produce anaerobic condition. The species which utilizes carbohydrates both fermentative and oxidative tube produces an acid reaction .
- **Lysine decarboxylase dehydrolase test:** Isolated colonies were stab inoculated into Moeller's decarboxylase medium with Lysine and overlaid with a 5mm layer of sterile paraffin oil, incubated at 37^{o}Cfor 24 hrs. Violet discoloration of the medium denotes positive reaction and yellow discoloration as negative reaction.
- **Nitrate reduction test:** Nitrate reduction broth was inoculated with a young agar slope culture and incubated at 37^{o}C for 96hours. After incubation 0.1ml of a mixture containing alpha napthylamine and sulfanilic acid in equal proportion was added. The development of red color within a few minutes indicates the ability of the organism to reduce nitrate.
- **Growth at variable temperature:** the culture from a young agar slope was inoculated onto two Nutrient agar plates and was incubated at 37^{o}Cand at 10˚ respectively. The presence / absence of growth at two different temperatures was used in the species identification.

Species	Indole	Gas	VP	Cit	ure	Lac	Mal	Lys
Klebsiella pneumonia Subsp aerogenes	-	+	+	+	+	+	+	+
Klebsiella pneumonia Subsp pneumoniae	-	+	-	+	+	+	+	+
Klebsiella pneumonia subsp ozaenae	-	*V*	-	*V*	-	*V*	-	*V*
Klebsiella pneumonia subsp rhinoscleromatis	-	-	-	-	-	+	-	-
Klebsiella oxytoca	+	+	+	+	+	+	+	+

Maintenance and preservation of culture strains:

Organisms grown in appropriate media for 18 hours were preserved in a nutrient agar slant at 2-8º C in a refrigerator and this culture was used within two weeks for routine laboratory works. For long term preservation, strains were stored in brain heart infusion broth with 20% glycerol and stored frozen without significant loss of viability at -20^{o}C until further study (Cheesbrough 2006).

ANTIMICROBIAL SENSITIVITY TESTING:

Disc diffusion method:

Antibiotic sensitivity was performed for all the isolates by Kirby- Bauer disc diffusion method using cation adjusted Mueller – Hinton agar plates. Three to four colonies were suspended in nutrient broth and were incubated for two hours at 37^{0}C, so as to get the organism in the logarithmic phase. The density of the

suspension was standardized with nutrient broth, visually equivalent 0.5 McFarland units. Within fifteen minutes of preparation of the suspension , a sterile cotton – wool swab was dipped into the suspension and the surplus was removed by rotating the swab against the side of the test tube. With this swab, the agar plate was inoculated by even streaking of the swab over surface of the plate in three directions so as to obtain a lawn culture. After brief drying, the antibiotic disc was placed, 5 on each plate. All the batches of antibiotics were quality checked as per standard guidelines. The control strains were included as per the CLSI guidelines.

The panel of drugs used for antimicrobial sensitivity testing were:-

ANTIBIOTICS	**RESISTANT (mm)**	**INTERMEDIATE (mm)**	**SENSITIVE (mm)**
Cefotaxime (30µg)	≤22	23-25	≥26
Cefepime (30µg)	≤18	19-24	≥25
Ceftazidime(30µg)	≤17	18-20	≥21
Piperacillin-Tazobactam(100/10µg)	≤17	18-20	≥21
Amikacin (30µg)	≤14	15-16	≥17
Gentamycin (10µg)	≤12	13-14	≥15
Ciprofloxacin(5µg)	≤15	16-20	≥21
Trimethoprim/Sulfamethoxazole (1.25/23.75µg)	≤10	18-20	≥16
Imipenem(10µg)	≤19	20-22	≥23
Ertapenem(10µg)	≤19	20-22	≥23

- Interpretations were made using the Clinical and Laboratory Standards Institute, USA guidelines (January 2016, M100-S24- Volume 34 No.1, Table 2B-2, page 62/63)

Minimum inhibitory concentration(MIC) by Epsilometer (E-test) method:

A predefined stable antimicrobial (Imipenem) gradient is present on a thin inert non -porous plastic carrier strip 5mm wide,60mm long known as Imipenem E-test strip.when this IMP E test strip is applied on to an inoculated agar plate , there is an immediate release of the drug and establishment of an antimicrobial concentration gradient in an agar medium. After overnight incubation,the tests are read by viewing the strips from the top of the plate,a symmetrical inhibition ellipse is produced.The intersection of the lower part of the ellipse shaped growth inhibition area with the test strip indicates the MIC value.The same MIC interpretative criteria used for dilution methods,as provided in CLSI guidelines are used with theE-strip value to assign an interpretive category of susceptible,intermediate,or resistant.

MIC INTERPRETATIVE CRITERIA FOR IMIPENEM.

CATEGORY	MIC VALUE(µg/ml)
SUSCEPTIBLE	≤1
INTERMEDIATE	2
RESISTANT	≥4

DETECTION OF ANTIMICROBIAL RESISTANCE MECHANISMS:

Phenotypic screening methods:

All the isolates which were included in this study were subjected to ESBL screening test using cefotaxime(30µg,zone size≤ 22mm) and ceftazidime (30µg, zone size ≤ 17mm) discs, AmpC screening test by Disc antagonism method using cefoxitin(30µg,zone size≤ 14mm) disc,Carbapenamase screening test using imipenem(10µg zone size ≤ 19mm)disc.The screening test positive isolates were subjected to respective confirmatory tests using appropriate antibiotic discs that were quality checked.

ESBL Detection by CLSI phenotypic confirmatory method:

In this method a lawn culture of the test isolates was made as for disc diffusion procedure. cefotaxime(30µg), Cefotaxime clavulanic acid disc (30/10µg),and ceftazidime(30µg) Ceftazidime clavulanic acid disc (30/10µg) (Himedia))were placed with in 20-24mm on the surface of the plate.The test isolates were considered to produce ESBL if the zone size around the beta lactamase inhibitor combination disc was increased by $\geq 5mm$ than with out the beta lactam inhibitor.The test was performed with appropriate controls.

Quality control:

Positive control - K.pneumoniae ATCC 700603

Negative control - E.coli ATCC 25922

AmpC Detection by EUCAST phenotypic confirmatory method:

In this method a lawn culture of the test isolates was made as for disc diffusion procedure. cefoxitin(30µg zone size≤ 19 mm)(Himedia)and Cefoxitin-cloxacillin disc (30/200µg) (Himedia)were placed between 20-24mm distance on the surface of the plate.The test isolates were considered to produce AmpC if the zone size around the inhibitor combination disc was increased by $\geq 5mm$ than with out inhibitor drug.The test was performed with appropriate control.

Carbapenamase detection by CLSI Modified Hodge Test(MHT):

In CLSI guidelines MHT is used for detection of Carbapenamase enzyme production. A 0.5 McFarland standard suspension of E.coli ATCC 25922(the indicator organism) from an overnight culture was prepared and was diluted 1:10 in saline or broth. The MHA plate was inoculated with the suspension by lawn culture and allowed to dry for 3 to 10 minutes. The 10µg of Ertapenem disc (Himedia) was placed in the centre. The test isolate was then streaked from the edge of the disc to the periphery of the plate along with positive and negative controls and incubated at 37^{o}C for 24 hours. The length of the streak should be 20 to 25mm.

Interpretation:

Enhanced growth of the test strain towards the zone of inhibition – positive for carbapenamase production. No enhanced growth of the test strain towards the zone of inhibition – negative for carbapenamase production.

Metallo beta lactamase Detection by EUCAST phenotypic method:

In this method a lawn culture of the test isolates was made as for disc diffusion procedure imipenem (10µg) (Himedia) and imipenem EDTA disc (10/750µg) (Himedia) were placed between 20-24mm distance on the surface of the plate.The test isolates were considered to produce MBL if the zone size around the inhibitor combination disc was increased by $\geq 7mm$ than without inhibitor drug.The test was performed with appropriate controls.

Klebsiella pneumonia carbapenamase Detection by EUCAST phenotypic method:

In this method a lawn culture of the test isolates was made as for disc diffusion procedure.Two Ertapenem disc (10µg),(Himedia)were placed 20-24mm distance on the surface of the plate. 10 µl of phenyl boronic acid solution (200µg) was added to one of the Ertapenem disc .The plate was incubated at 37˚ for 24 hrs.The test isolates were considered to produce KPC if the zone size around the inhibitor combination disc was increased by $\geq 5mm$ than without boronic acid . The test was performed with appropriate controls.

MOLECULAR METHOD:

Polymerase chain reaction:

The isolates which were resistant to imipenem by Kirby bauer Disc diffusion method which positive for both MHT and KPC phenotypic detection method were subjected to RT-PCR for the detection of bla KPC gene.

DNA extraction:

5-10 Klebsiella colonies were inoculated into nutrient broth and incubated overnight at 37º C.1.5 ml of overnight broth culture are transferred into 2.5ml of centrifuge tube and centrifuged at 10,000 rpm for 3 minutes.Supernatant was discarded,excess medium was removed by gentle tapping the tube on a paper towel.

Procedure:

1) The pellet obtained was suspended in 200µl of PBS.
2) 180µl of Lysozyme digestion buffer and 20µl of Lysozyme were added.
3) Above mixture was mixed well and incubated at 37º C for 15 min.
4) After incubation 200µl of Lysis buffer and 20µl of Proteinase K (10mg/ml) were added and incubated at 56º C for 10 min in water bath.
5) Then 300µl of Isopropanol was added and mixed well.
6) The whole lysate was transferred into PureFast spin column and centrifuged at 10000 rpm for 1 min.
7) Flow through was discarded and 500µl of Wash buffer -1 was added to spin column and centrifuged at 10000 rpm for 1 min.
8) Flow through was discarded and 500µl of Wash buffer -2 was added to spin column and centrifuged at 10000 rpm for 1 min.washing was repeated one more time.

9) Flow through was discarded and the spin column was centrifuged for additional 2 minutes to remove any residual ethanol.

10) The DNA was eluted by adding 100µl of Elution buffer and centrifuged for 1 min.The eluted DNA was used as the template for PCR.

Primers:(Designed by HELINI Biomolecules,Chennai)

GENE	PRIMER SEQUENCES	AMPLICON SIZE
KPC-FORWARD PRIMER	5'-CGGCAGCAGTTTGTTGATTG-3'	882 bp
KPC –REVERSE PRIMER	3'-CGCTGTGCTTGTCATCCTTG-5'	

PCR Procedure:

1.Reactions were set up as follows;

COMPONENTS	QUANTITY
HELINI Red Dye PCR Master mix	10µl
HELINI Ready to use-bla KPC gene primer mix	5µl
Purified Bacterial DNA	5µl
Total volume	20µl

2. Mixed gently and spin down briefly.

3. Place into PCR machine (Corbett Research thermocycler) and program it as follows;

Cycle number	Denaturation	Annealing	Extension.
1	94º C for 5 min.	-	-
35	94º C for 30 sec	58º C for 30 sec	72º C for30 sec
1	-	-	72º C for 5 min.

Loading:

1. Prepare 2% agarose gel.(2 gm of agarose in 100 ml of IX TAE buffer)
2. Run electrophoresis at 50 v till dye reaches three fourth distances and observe the bands in UV Transilluminator.

Interpretation:

The amplified PCR products and 100 bp DNA molecular markers wers seen as bright fluorescent bands with statisfactory controls. A 882bp-989bp corresponds to bla KPC gene

Results

RESULTS

This cross sectional study was conducted in the Institute of Microbiology,Madras Medical College in association with various other Departments at the Rajiv Gandhi Government General Hospital,Chennai during the period of Sep2016-Aug 2017. A total of 200 clinically signicant ,consecutive ,nonduplicate isolates of Multidrug resistant Klebsiella species from various clinical specimens were included in the study.All the isolates were identified by standard procedures.

TABLE-1:GENDER AND AGE DISTRIBUTION OF THE PATIENTS (n=200)

AGE IN YEARS	NO.OF PATIENTS			PERCENTAGE
	MALE	FEMALE	TOTAL	
18-20	18(12.6%)	17(11.9%)	35	17.5%
21-40	42(29.5%)	19(32.6%)	61	30.5%
41-60	70(49.2%)	19(32.6%)	89	44.5%
>60	12(8.4%)	3(5.1%)	15	7.5%
TOTAL	142(71%)	58(29%)	200	100%

There was a male predominance(71%) among the isolates obtained from the patients.The maximum number of isolates were from the patients in the age group of 41-60 yrs(44.5%) followed by 21-40yrs(30.5%).

FIGURE -1:GENDER AND AGE DISTRIBUTION OF THE PATIENTS (n=200)

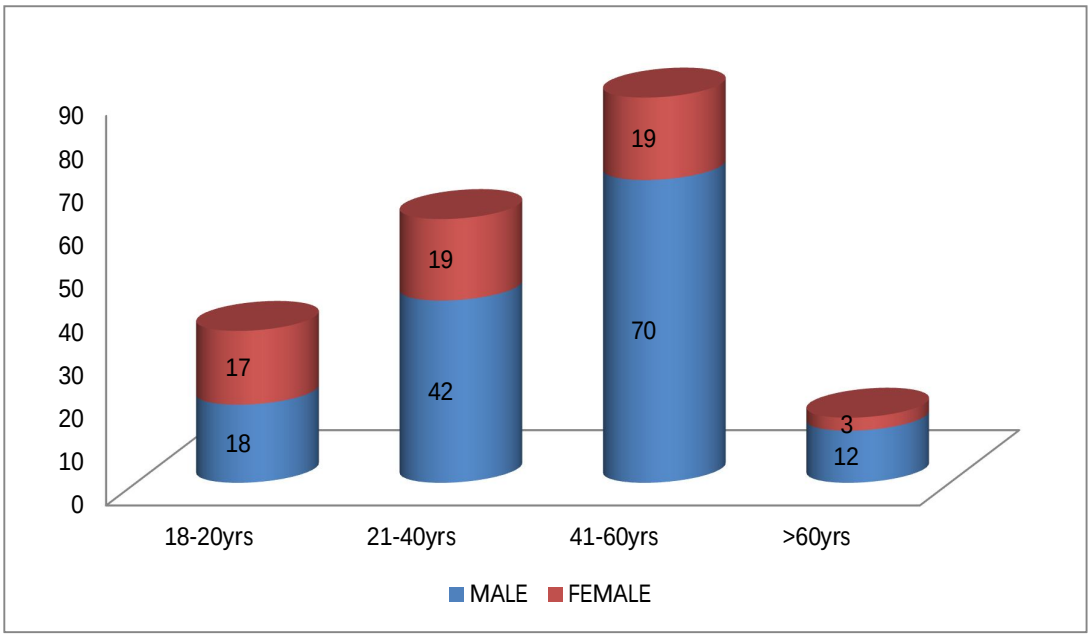


TABLE-2:DISTRIBUTION OF KLEBSIELLA ISOLATES FROM VARIOUS CLINICAL SPECIMENS (n=200)

CLINICAL SAMPLES	ISOLATES	PERCENTAGE
PUS	76	38%
URINE	49	24.5%
SPUTUM	24	12%
BODY FLUIDS	19	9.5%
TRACHEAL ASPIRATES	14	7%
DEVICES	12	6%
CSF	3	1.5%
BLOOD	3	1.5%
TOTAL	200	100%

Most of the Klebsiella isolates obtained were from pus samples(38%) followed by urine samples(24.5%) followed by sputum (12%),body fluids(9.5%),tracheal aspirates(7%),devices(6%),CSF(1.5%),blood(1.5%).

FIGURE -2:DISTRIBUTION OF KLEBSIELLA ISOLATES FROM VARIOUS CLINICAL SPECIMENS (n=200)

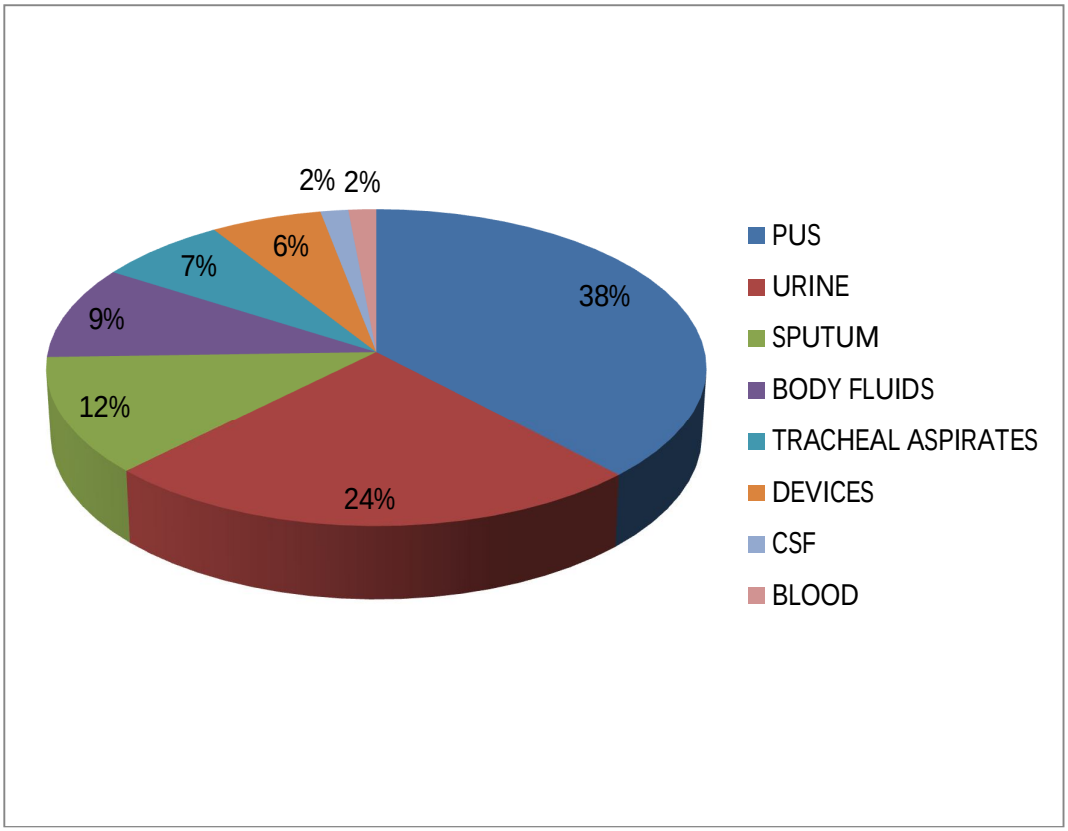


TABLE-3: DISTRIBUTION OF MULTIDRUG RESISTANT KLEBSIELLA ISOLATES IN VARIOUS CLINICAL SPECIMENS FROM DIFFERENT WARDS (n=200).

SPECIALITY		SPECIMENS	NUMBER OF ISOLATES	TOTAL	PERCENTAGE
ICU	IMCU	Urine	8	58	29%
		Tracheal aspirates	7		
		Catheter tip	5		
		Sputum	2		
		Blood	2		
	ISCU	Pus	9		
		Wound swab	6		
		Urine	3		
		Drainage tube	3		
		Catheter tip	1		
	URO-ICU	Urine	8		
		PD fluid	2		
		Pus	1		
		Blood	1		
ORTHOPEDICS WARD		Pus	19	33	16.5%
		Woundswab	10		
		Urine	4		
SURGERY WARD		Pus	17	27	13.5%
		Wound swab	6		
		Urine	2		
		Drainage tube	2		
URO/NEPHRO		Urine	20	26	13%
		PD fluid	42		
		Pus	2		
THORACIC MEDICINE		Sputum	18	24	12%
		Bronchial wash.	6		
NEUROSURGERY		Tracheal aspirates	7	17	8.5%
		Wound swab	3		
		CSF	3		
		Urine	2		
		Pus	1		
		Catheter tip	1		
MEDICINE WARD		Sputum	4	13	6.5%
		Ascitic fluid	4		
		Pleural fluid	3		
		Urine	2		
		Others.	3	3	1.5%
TOTAL				**200**	**100%**

TABLE-4:DISTRIBUTION OF ASSOCIATED RISK FACTORS

RISK FACTORS	NUMBER OF ISOLATES.
ICU STAY	58
PREVIOUS H/O HOSPITALISATION	42
DIABETES MELLITUS	37
SURGERY/TRAUMA	30
CHRONIC ILLNESS(TB/COPD)	20
ALCOHOLISM/CHR.LIVER DISEASE	7
MALIGNANCY	6

Majority of the multidrug resistant Klebsiella isolates were from the Intensive care unit followed by other high risk associated factors like previous hospitalisation,diabetes,and surgery/ trauma.

FIGURE-3 :DISTRIBUTION OF ASSOCIATED RISK FACTORS

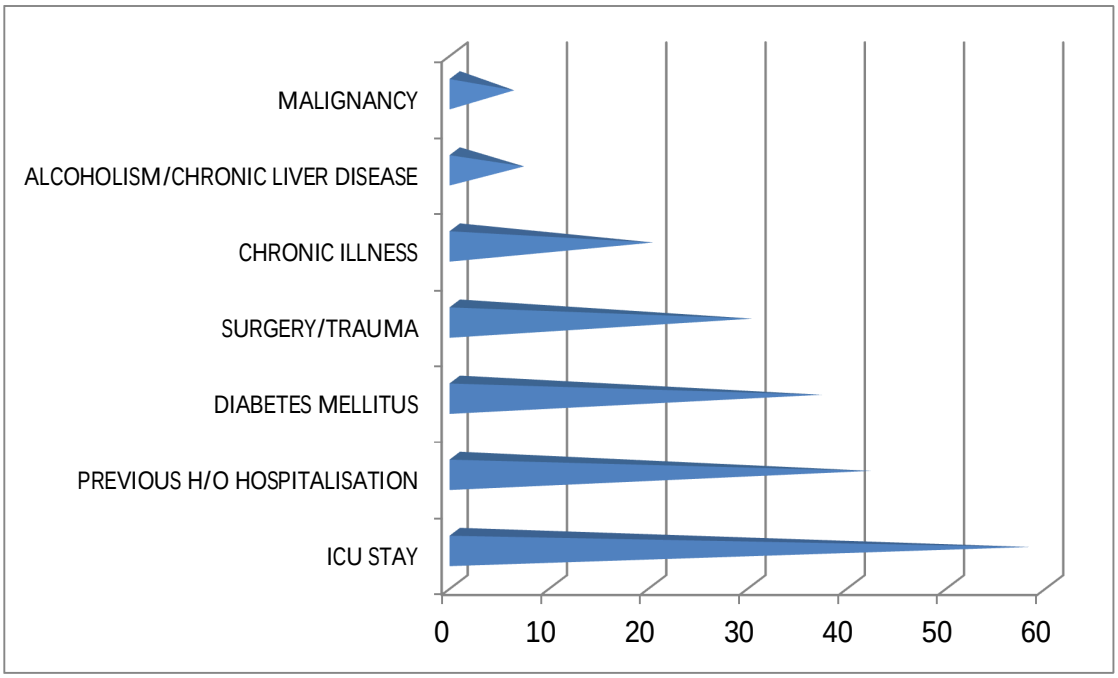


TABLE-5: DISTRIBUTION OF KLEBSIELLA SPECIES (n=200)

S.NO	SPECIES	NUMBER OF ISOLATES	PERCENTAGE
1	Klebsiella pneumonia subsp aerogenes.	96	48%
2	Klebsiella oxytoca	92	46%
3	Klebsiella pneumonia subsp pneumonia.	12	6%
	TOTAL	200	100%

Klebsiella pneumoniae subsp aerogenes(48%) was the most common species isolated followed by Klebsiella oxytoca(46%) and K.pneumoniae subsp pneumonia(6%).

FIGURE -4: DISTRIBUTION OF *KLEBSIELLA* SPECIES.(n-200)

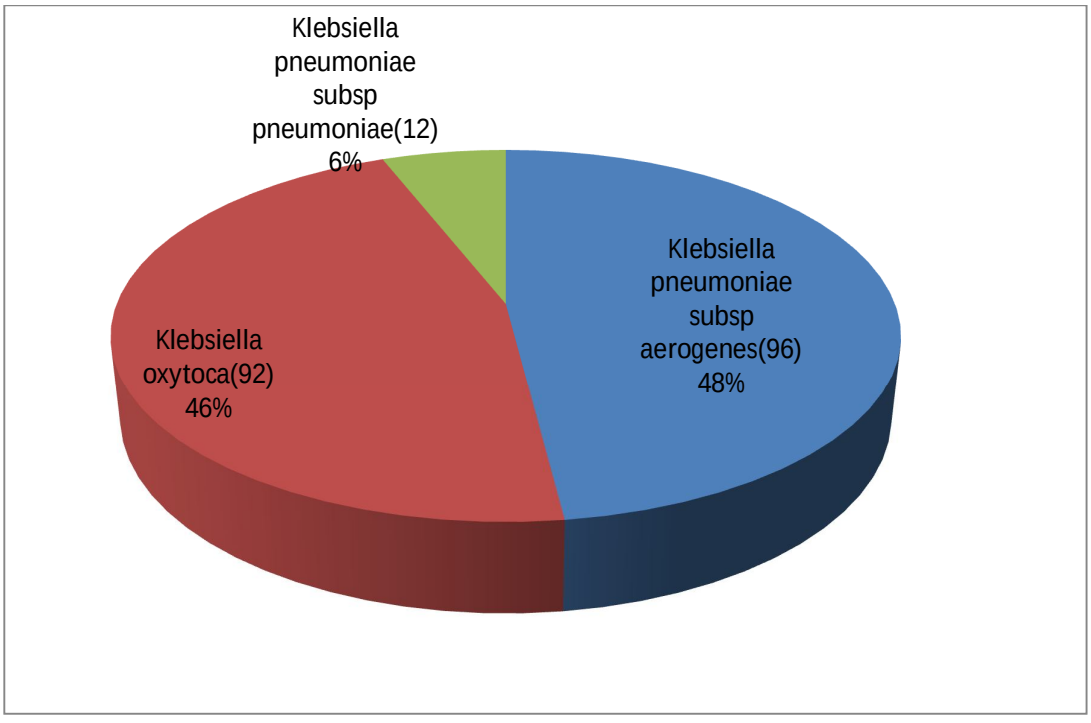


TABLE-6: DISTRIBUTION OF KLEBSIELLA SPECIES IN VARIOUS CLINICAL INFECTIONS (n=200)

Infections	Klebsiella pneumonia subsp aerogenes	Klebsiella oxytoca	Klebsiella pneumoniae subsp pneumonia
Wound infections	32	34	2
Respiratory infections	19	21	6
Chronic illness (CKD,CLD	20	21	2
Urinary tract infections	18	14	2
Hydrocephalus	4	3	-
Septicemia	3	-	-

Both Klebsiella species(Klebsiella pneumonia subsp aerogenes, and klebsiella oxytoca) were most commonly isolated in wound infections followed by respiratory infections and urinary tract infections.But *Klebsiella pneumonia subsp pneumoniae* were isolated most commonly in respiratory tract infections followed by others.

FIGURE-5: DISTRIBUTION OF KLEBSIELLA SPECIES IN VARIOUS CLINICAL INFECTIONS(n=200)

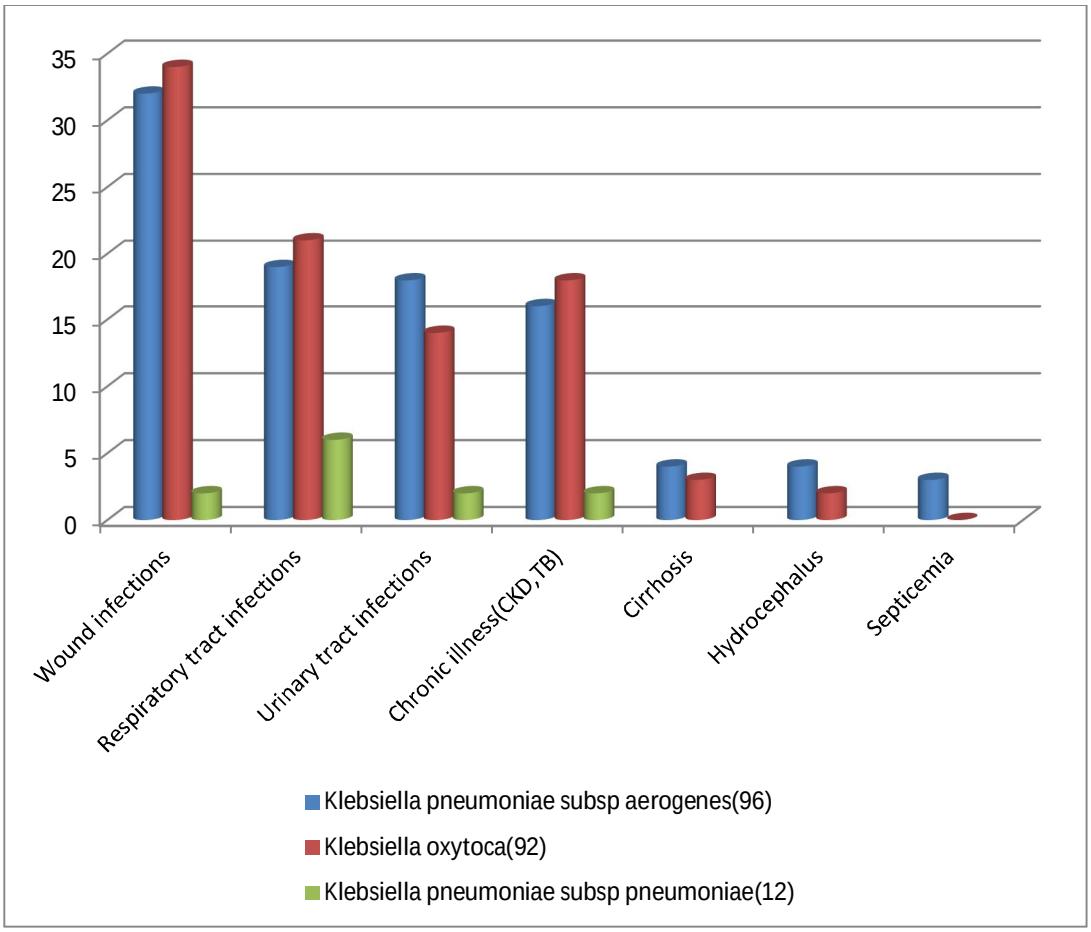


TABLE -7 : ANTIBIOTIC SUSCEPTIBILITY PATTERN(N=200)

ANTIBIOTICS	Klebsiella pneumonia		Klebsiella oxytoca	
	Number	Percentage	Number	Percentage
COT	68	63%	60	65%
CIP	71	66%	38	41%
NOR (urine-K.p-19 K.o-30)	15	79%	18	60%
NIT (urine-K.p-19 K.o-30)	15	79%	24	80%
GEN	52	48%	62	67%
AK	86	80%	64	70%
CTX	18	16%	10	11%
P/T	55	51%	63	68%
IPM	72	67%	73	79%
ETP	84	78%	71	77%

FIGURE-6:ANTIBIOTICS SUSCEPTIBILITY PATTERN OF KLEBSIELLA ISOLATES (n=200)

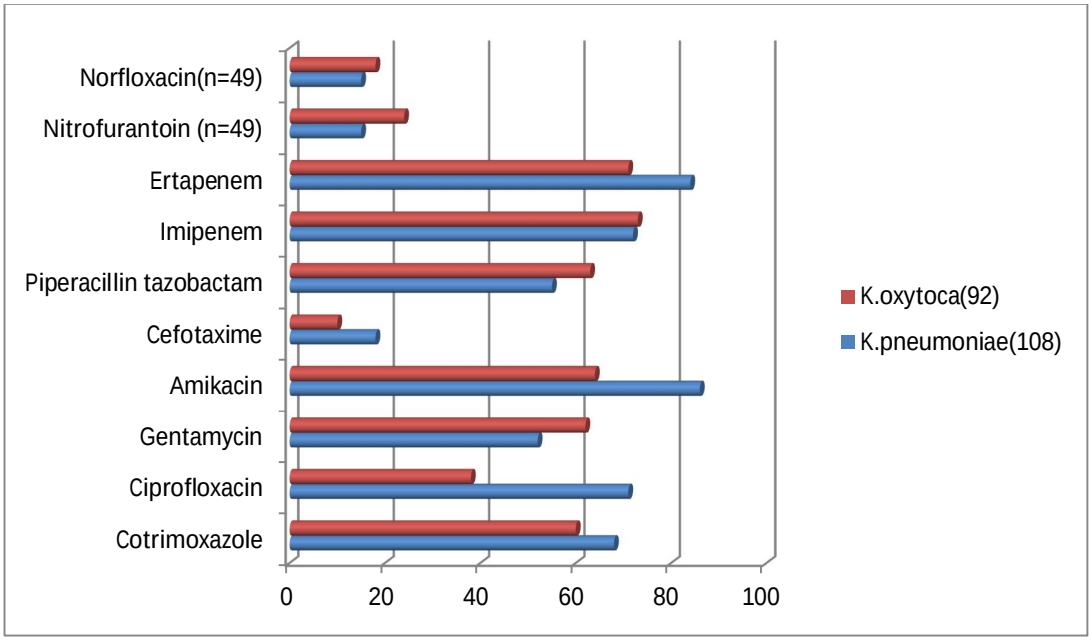


TABLE -8: COMPARISON OF ANTIMICROBIAL SENSITIVITY PATTERN AMONG KLEBSIELLA SPECIES(n=200)

DRUGS	KLEBSIELLA PNEUMONIAE (n=108)		KLEBSIELLA OXYTOCA (n=92)		TEST	P VALUE	SIGNIFICANCE
	SEN	RES	SEN	RES			
Cefotaxime	16	92	11	81	Chi-square	0.035	Yes
Ciproflox	66	42	38	54	Chi-square	0.005	Yes
Cotrimoxazole	68	40	60	32	Chi-square	0.043	Yes
Gentamycin	52	56	62	30	Chi-square	0.006	Yes
Amikacin	86	22	64	28	Chi-square	0.011	Yes
Piperacillin-Tazobactam	55	53	63	29	Chi-square	0.012	Yes
Imipenem	72	36	73	19	Chi-square	0.045	Yes

Data were analysed using SPSS software (Version 10.0; SPSS Inc., Chicago). Chi-square and Fisher's exact test was performed to determine statistically significant differences among the antibiotic susceptibility rate of *K. pneumonia e and Klebsiella oxytoca* isolates. The standard significance level, $P < 0.05$, was used, and all tests of statistical significance were two-tailed. There was a significant difference between the antimicrobial sensitivity pattern of *Klebsiellapneumonia* and *Klebsiella oxytoca* since p value is < 0.05 for cephalosporin ,aminoglycosides,quinolones and carbapenems .

TABLE-9:DETECTION OF IMIPENEM RESISTANCE IN KLEBSIELLA SPECIES BY DISC DIFFUSION METHOD(N=200)

IMIPENEM SUSCEPTIBILITY	DISC DIFFUSION METHOD	PERCENTAGE
SUSCEPTIBLE	145	72.5%
RESISTANT	55	27.5%

Among the 200 isolates of Klebsiella species were screened for Imipenem resistance by Kirby-Bauer disc diffusion method of which 55 isolates(27.5%) were found to be resistant to imipenem.

FIGURE-7:DETECTION OF IMIPENEM RESISTANCE IN *KLEBSIELLA SPECIES* BY DISC DIFFUSION METHOD (N=200)

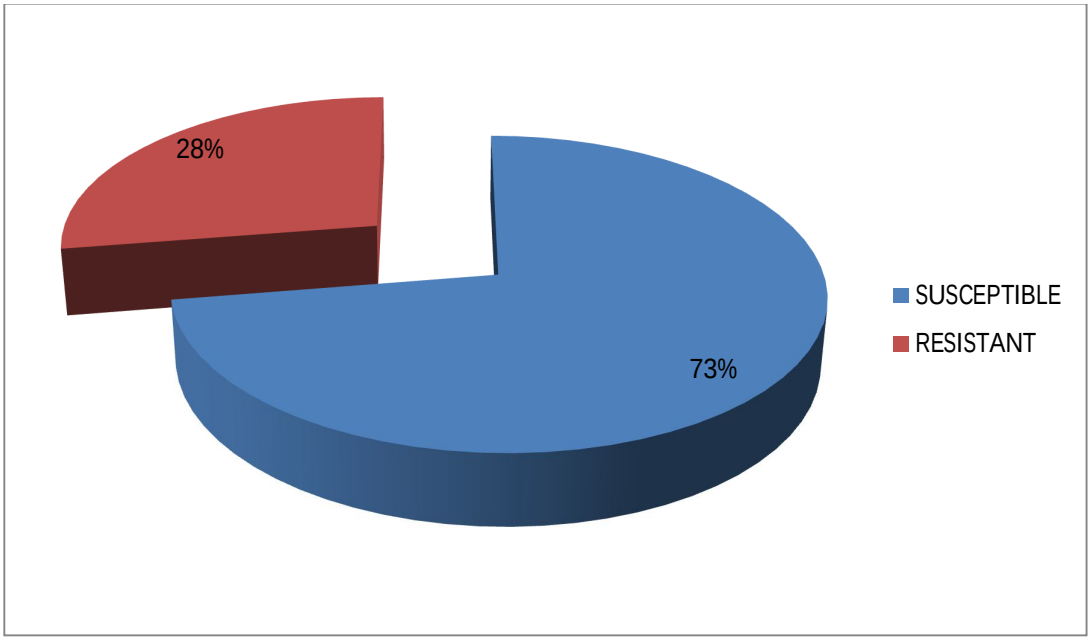


TABLE-10: MIC FOR IMIPENEM RESISTANT ISOLATES (n=55).

Mic For Imipenem (ug/ml)	32	16	8	4	2	1	0.5	0.25
Klebsiella pneumoniae subsp aerogenes (32)	20	7	2	3	-	-	-	-
Klebsiella oxytoca (23)	13	6	2	2	-	-	-	-

MIC INTERPRETATIVE CRITERIA FOR IMIPENEM.

CATEGORY	**MIC VALUE(µg/ml)**
SUSCEPTIBLE	≤1
INTERMEDIATE	2
RESISTANT	≥4

Among the 55 isolates were found to be resistant to Imipenem by Double dics diffusion method which was confirmed by MIC by E-strip method..Among the 55 isolates,33 isolates had 32(µg/ml) as MIC,13 isolates had 16(µg/ml) as MIC,4 isolates had 8(µg/ml) as MIC,and remaining 5 had 4(µg/ml) as MIC

TABLE-11:DETECTION OF EXTENDED SPECTRUM BETALACTAMASES AMONG KLEBSIELLA SPECIES(N=119)

ISOLATES	CEFOTAXIME (CTX30µg)	CTX + CLAVULANIC ACID (30+10µg)	CEFTAZIDIME (CAZ-30µg)	CAZ + CLAVULANIC ACID (30+10µg)	ESBL PERCENTAGE
Klebsiella pneumoniae subsp aerogenes	81	59	81	59	49.6%
Klebsiella pneumoniae subsp pneumonia	7	3	7	7	2.5%
Klebsiella oxytoca	82	57	82	82	47.9%
TOTAL	170	119	170	119	100%

Among the 200 isolates of Klebsiella species were screened for ESBLproduction and confirmed by CLSI phenotypic confirmatory method.59(49.6%) isolates of K.pneumoniae subsp aerogenes,57(47.9%) isolates of K.oxytoca and 3(2.5%) K.pneumoniae subsp pneumoniae were found to be ESBL producers.

FIGURE-8 : DETECTION OF EXTENDED SPECTRUM BETALACTAMASES AMONG KLEBSIELLA SPECIES(N=119)

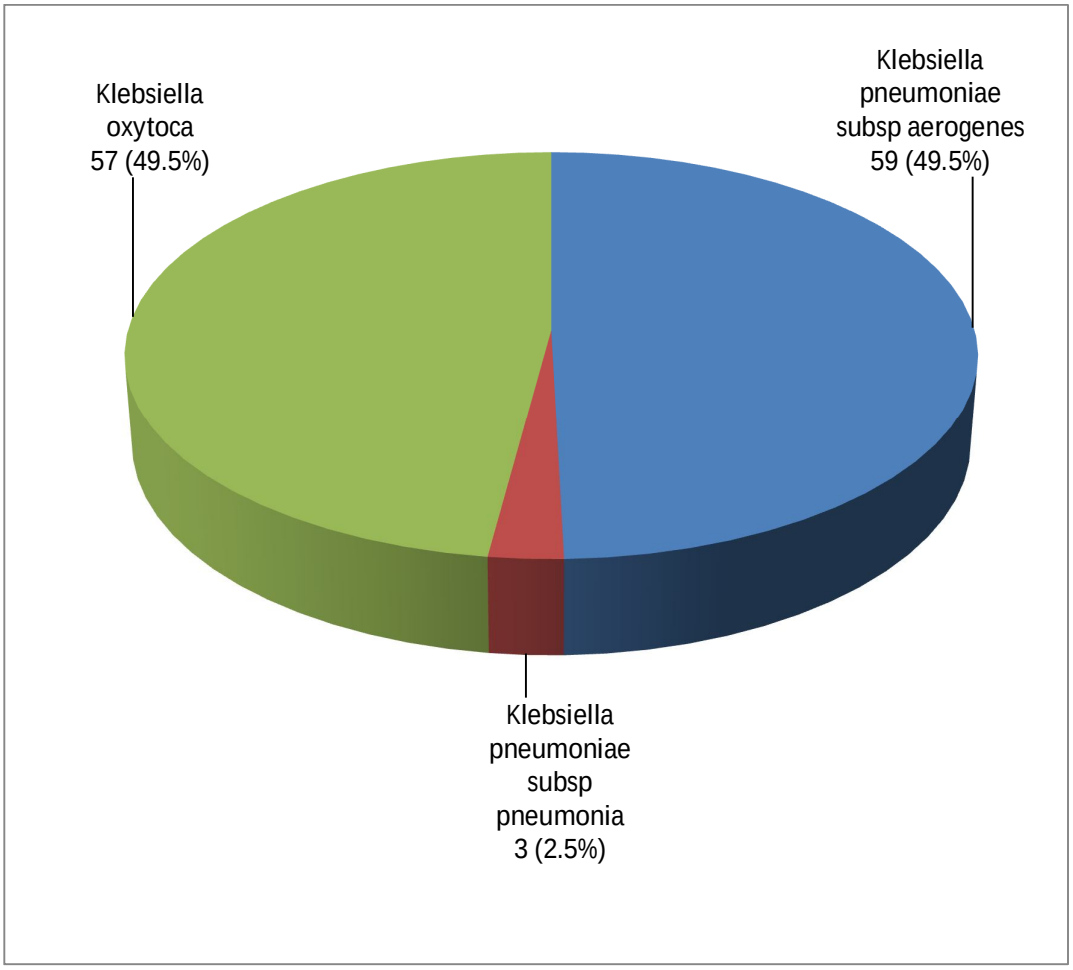


TABLE-12 : DETECTION OF AMPC BETALACTAMASES AMONG *KLEBSIELLA SPECIES(N*=13)

SPECIES	CEFOXITIN (30µg) ≤22mm	CEFOXITIN + CLOXACILLIN (30+200µg) ≥27mm	PERCENTAGE
Klebsiella pneumoniae subsp aerogenes (96)	46	8	61.5%
Klebsiella oxytoca (92)	48	5	38.5%
TOTAL	92	13	100%

The AmpC beta lactamases were common in K.p.subsp aerogenes(61.5%) and K.oxytoca(38.5%).

FIGURE 9 : DETECTION OF AMPC BETALACTAMASES AMONG *KLEBSIELLA SPECIES(N*=13)

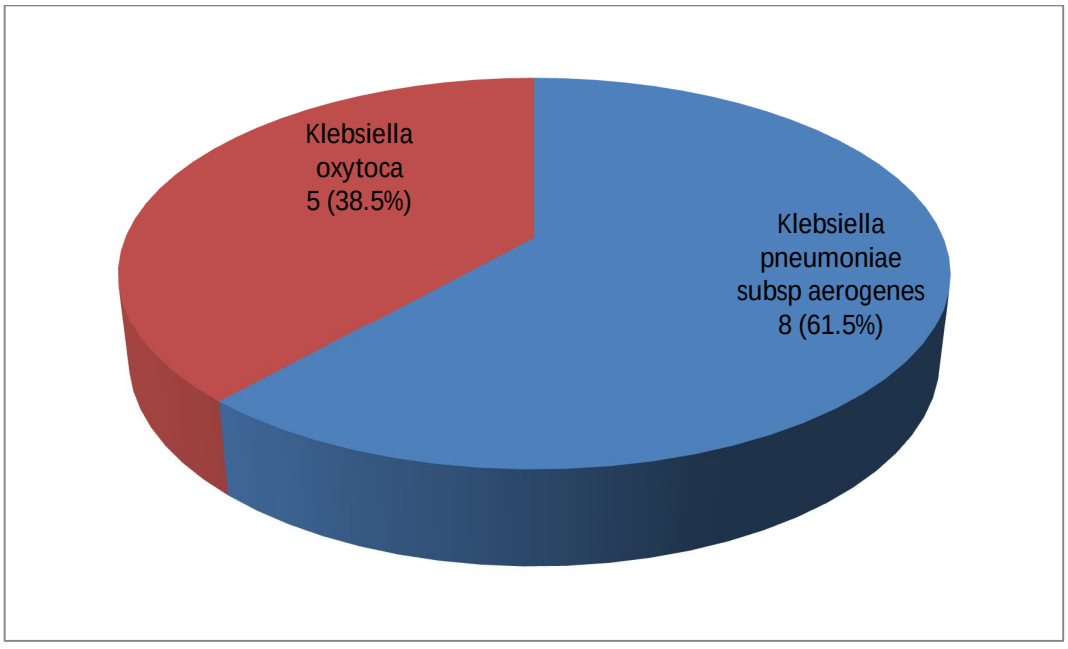


TABLE-13:DETECTION OF KLEBSIELLA PNEUMONIAE CARBAPENAMASES AMONG KLEBSIELLA SPECIES. (N=12).

SPECIES	ERTAPENEM (ETP 10µg)	ETP+PBA (10+200µg)	KPC PERCENTAGE
Klebsiella pneumoniae subsp aerogens (96)	35	8	66.7%
Klebsiella oxytoca(92)	25	4	33.3%
TOTAL	55	12	100%

The Klebsiella pneumonia carbapenamase were common in K.p.subsp aerogenes(66.7%) and K.oxytoca(33.3%).

FIGURE-10: DETECTION OF KLEBSIELLA PNEUMONIAE CARBAPENAMASES AMONG *KLEBSIELLA* species.

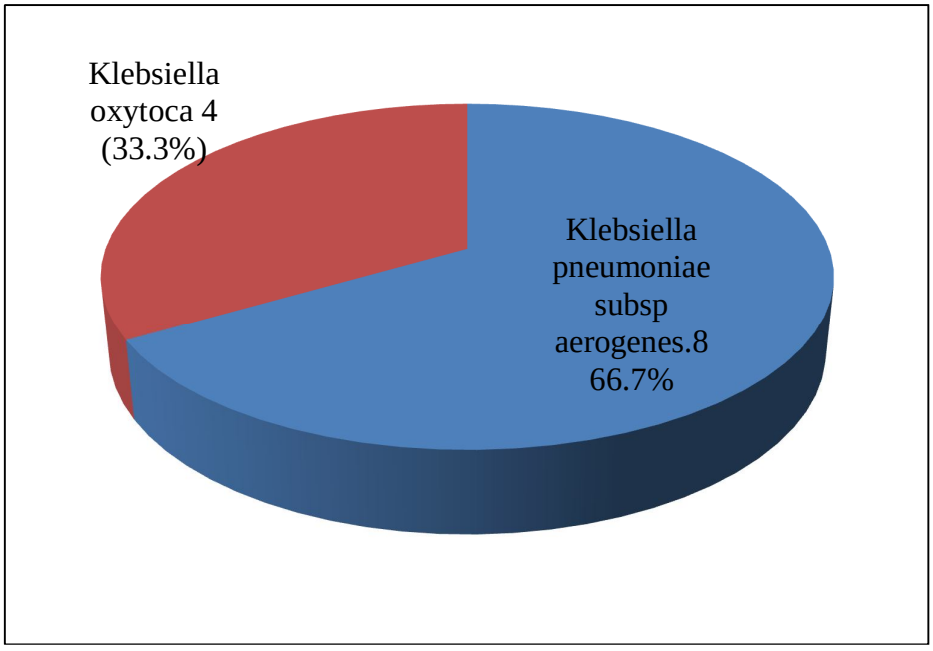


TABLE-14 : DETECTION OF METALLOBETALACTAMASES AMONG KLEBSIELLA SPECIES(N=16)

SPECIES	IMIPENEM (IMP 10µg)	IPM+EDTA (10+750µg)	MBL PERCENTAGE
Klebsiella pneumoniae subsp aerogens (96)	35	6	37.5%
Klebsiella oxytoca(92)	25	10	62.5%
TOTAL	55	16	100%

The Metallobetalactamase were common in K.oxytoca(62.5%) and K.pneumoniae subsp aerogenes(37.5%).

FIGURE 11 : DETECTION OF METALLOBETALACTAMASES AMONG KLEBSIELLA SPECIES (N=16)

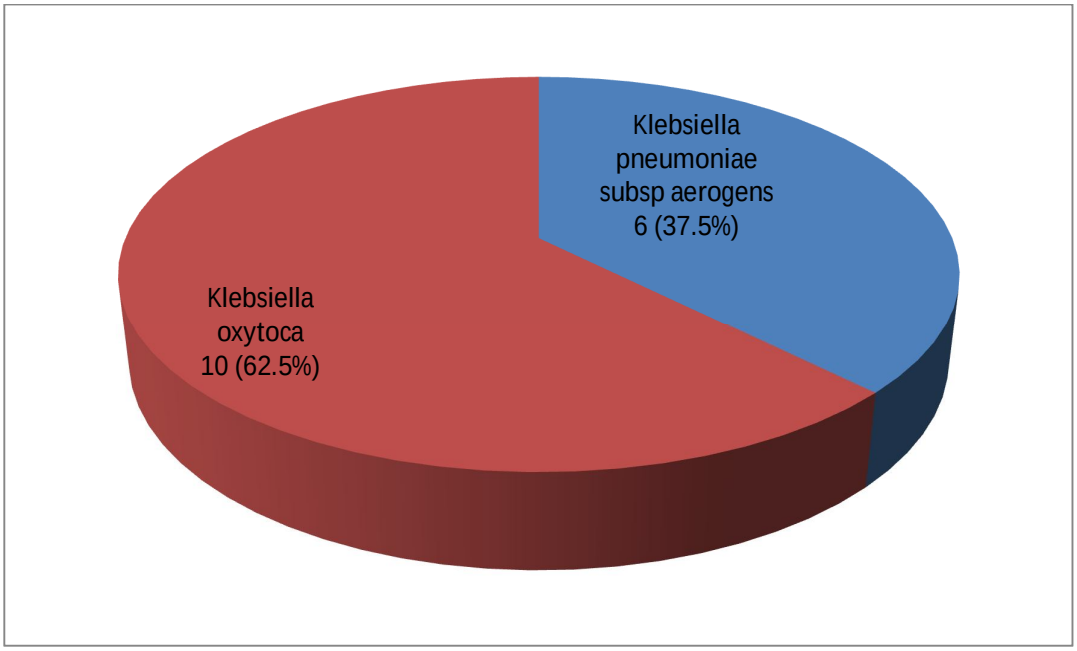


TABLE-15 : DETECTION OF ENZYME CO PRODUCERS AMONG KLEBSIELLA SPECIES (N=25)

ENZYME CO PRODUCERS	ISOLATES		TOTAL	PERCENTAGE
	Klebsiella pneumonia subsp aerogenes	Klebsiella oxytoca		
ESBL+AMP C	8	2	10	40%
ESBL +MBL	2	1	3	12%
MBL+KPC	5	4	9	36%
AMP C+MBL	2	1	3	12%
TOTAL	17	8	25	100%

The most common Enzyme coproducers ESBL+AMPC were common in K.p.subsp aerogenes and K.oxytoca followed by MBL+KPC which is more common in Klebsiella oxytoca.

FIGURE 12 : DETECTION OF ENZYME CO PRODUCERS AMONG KLEBSIELLA SPECIES (N=25)

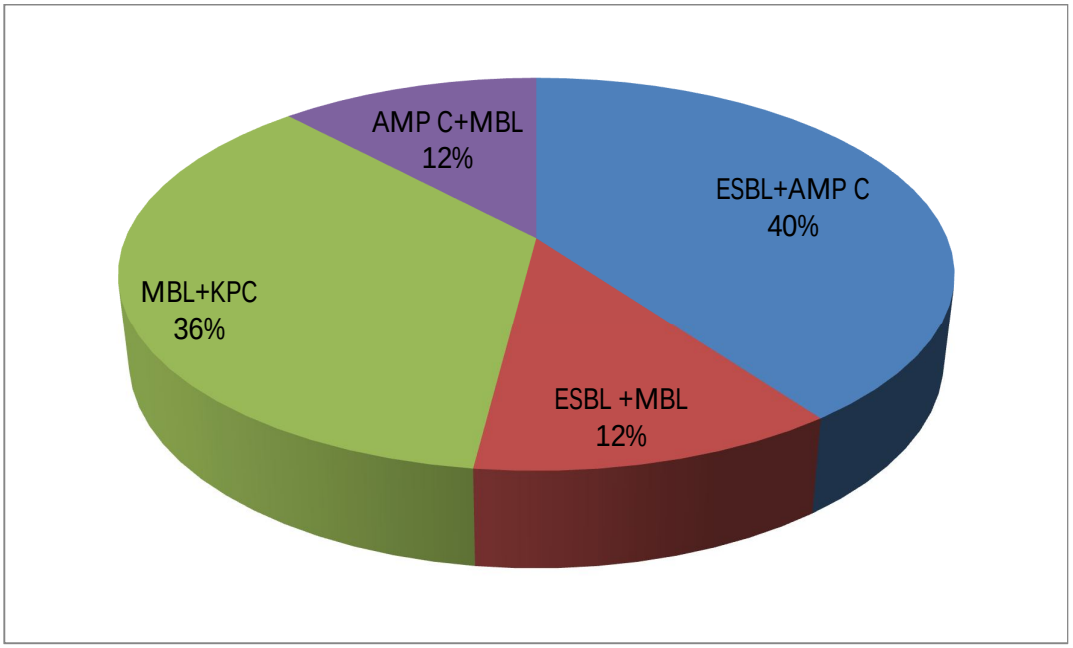


TABLE-16 : SPECTRUM OF RESISTANCE AMONG *KLEBSIELLA SPECIES* (N=200).

Drug Resistant Pattern	No.of Isolates.			Total	Percentage	Test	P value	Significance
	K.p.a	K.p.p	K.o					
ESBL	56	10	53	119	59.5%	Chi-square	0.016	Significant
Amp C	8	-	5	13	6.5%	Fisher's Exact	0.031	Significant
MBL	6	-	10	16	8%	Fisher's Exact	0.045	Significant
KPC	8	-	4	12	6%	Fisher's Exact	0.004	Significant
ESBL+ AmpC	6	2	2	10	5%	Chi-square	0.032	Significant
MBL+ KPC	5	-	4	9	4.5%	Fisher's Exact	0.001	Significant
AmpC+MBL	2	-	1	3	1.5%	Fisher's Exact	0.001	Significant
ESBL+ MBL	2	-	1	3	1.5%	Fisher's Exact	0.001	Significant
OTHER MECHANISM	3	-	12	15	7.5%	Fisher's Exact	0.78	Not Significant

Among the 200 isolates of Klebsiella species 119(59.5%) were ESBL producers,followed by MBL 16(8%) ,AmpC 13(6.5%) ,KPC 12(6%) Enzyme coproducers 25(12.5%s, other mechanism 15(7.5%). There was a significant difference between the antimicrobial resistance pattern of K.pneumoniae subsp aerogenes and other species.

TABLE-17 : MOLECULAR CHARACTERISATION OF KPC KLEBSIELLA ISOLATES.

MODIFIED HODGE TEST	KPC PHENOTYPIC CONFIRMATION	KPC GENOTYPIC CONFIRMATION (bla KPC gene)
40	16	12

Among the 40 MHT positive isolates ,all the 16 isolates were screened and phenotypically confirmed for Klebsiella pneumoniae carbapenamase by EUCAST Guidelines for resistance detection method .Among the 16 isolates KPC gene (blaKPC) positive for 12 isolates.

TABLE-18:CLINICAL OUTCOME OF THE PATIENTS WITH IMIPENEM RESISTANCE AND THEIR GENETIC MARKERS .

S. NO	AGE / SEX	WARD NO / IP NO	DIAGNOSIS	SPECIMEN	SPECIES	PHENO TYPE (ETP, ETP + PBA) (KPC)	GENE IDENTIFIED	RECOVERY OF PATIENT
1	60y/ F	44734/ TM-1	DM / Non resolving pneumonia.	Bronchial wash	Klebsiella pneumoniae subsp aerogenes	KPC	bla KPC	Expired
2	65y/ F	55041/ 231	LT .DFS.A/K amputation	PUS	Klebsiella oxytoca	KPC	bla KPC	Recovered
3	45Y/ F	63019/ Or-1V	D1D2 disc prolapsed.disc stabilisation	PUS	Klebsiella pneumoniae subsp aerogenes	KPC	bla KPC	Recovered
4	60Y/ M	42645 /254	Periampullary Ca of pancreas. Whipples procedure	BILE	Klebsiella pneumoniae subsp aerogenes	KPC	bla KPC	Expired
5	20Y/ M	67500/ NS-II	Post Meningitic Hydrocephalus	Tracheal aspirate	Klebsiella oxytoca	KPC	bla KPC	Recovered

S. NO	AGE / SEX	WARD NO / IP NO	DIAGNOSIS	SPECIMEN	SPECIES	PHENO TYPE (ETP, ETP + PBA) (KPC)	GENE IDENTIFIED	RECOVERY OF PATIENT
6	45Y/M	54856/30's	DFS LTFoot.LT Great Toe amputation	PUS	Klebsiella oxytoca	KPC	bla KPC	Recovered
7	40Y/F	59207/132	Degloving injury Rt thigh	PUS	Klebsiella pneumoniae subsp aerogenes	KPC	bla KPC	Recovered
8	60Y/F	50600/253	Ca.stomach-stage IIcorrective surgery	Tracheal aspirates	Klebsiella pneumoniae subsp aerogenes	KPC	bla KPC	Expired
9	65Y/M	65962/241	Chr.alcoholism/CLD	Asitic fluid	Klebsiella pneumoniae subsp aerogenes	KPC	bla KPC	Recovered
10	20Y/F	55887/162	SLE/pustules	PUS	Klebsiella pneumoniae subsp aerogenes	KPC	bla KPC	Recovered
11	52Y/M	64594/125	COPD/Acute exaerbation	SPUTUM	Klebsiella pneumoniae subsp aerogenes	KPC	bla KPC	Recovered
12	65Y/M	63756/47	BPH/ Chr.UTI.	URINE	Klebsiella oxytoca	KPC	bla KPC	Recovered

Among the 55 Imipenem resistant isolates only 12 isolates were positive for bla kpc gene .3 patients were Expired .The mortality rate here is 25% mortality rate were seen among the patients who had been infected with KPC producing Klebsiella pneumoniae.

Discussion

DISCUSSION

Now a days antibiotics have been used extensively and newer antibiotics are continuously being added for the treatment of various infections. An extensive use of β-lactam antibiotics in hospital and community has created a major problem leading to increased morbidity, mortality and health care costs[14]. Proper use of antibiotics is very important for various reasons. *Klebsiella* infections present a global medical challenge because it is an important opportunistic GNB in health care institutions.It has gained importance because of its ability to survive under a wide range of environmental conditions,having acquired drug resistance mechanisms and the emergence of multidrug and pan drug resistant strains. The isolation and identification of resistance pattern of *Klebsiella* infections helps in selection of appropriate antibiotics,reducing the morbidity and mortality of the patients and in reducing the spread of resistant strains in the community.

This cross sectional study was conducted in the Institute of Microbiology, Madras Medical College, Chennai during the period of Sep2016-Aug 2017. The present study includes 200 clinically significant, consecutive, non-duplicate *Multidrug resistant Klebsiella isolates.*

In the present study ,among the 200 *Klebsiella* isolates,142 (71%)isolates were from male patients and remaining 58 (29%) isolates were from female patients (Table-1).The male to female ratio 2.45:1 which is high when compared to study done by Sunilkumar Biradar et al where he reported male to female ratio

of1.7:1. .Out of 200 isolates 89 isolates (44.5%) were from the patients in the age group of 41-60yrs followed by 61 isolates(30.5%) in 21-40yrs age group 35 isolates (17.5%)from patients aged 18-20yrs,and 15 isolates(7.5%) in >60 yrs age groups.

In the present study, the distribution of *Klebsiella* species in various clinical specimens was in the following order,pus specimen 76(38%),urine 49(24.5%) sputum24 (12%),body fluids 19 (9.5%),Tracheal aspirates 14 (7%) devices12 (6%),CSF 3 (1.5%),blood 3 (1.5%)(Table-2).This is very similar to the study conducted by Sunilkumar Biradar et al.where50% of isolates were from pus specimens followed by urine(21%) where as Shalley Dahiya et.al**,**obtained Maximum isolates were from pus samples (21.55%) followed by throat swabs (18.42%), urine (15.59%), stool (9.37%), blood (9.18%).Where as Amit kumar singh et al, in his study reported that *Klebsiella* isolates were common from blood(65%) followed by sputum(58%)urine(53%) and pus(44%).

In this study, the Multidrug resistance *Klebsiella* isolates were isolated predominantly from patients in Intensive care units- (29%) followed by Orthopaedics ward(16.5%)Surgery ward(13.5%) Nephrology(13%) Thoracic medicine(12%) Neurosurgery(8.5%) Medicine (6.5%) and from other wards (1.5%) (Table-4). This is similar to the study conducted by Vemula Sarojamma et al where most of isolates were obtained from ICU (19%) followed by surgery and trauma(13%).

Major risk factor for colonisation or infection with Multidrug resistance organisms are long term antibiotic exposure, prolonged intensive care unit stay, nursing home residency, severe illness, residence in an institution with high rates of ceftazidime and other third generation cephalosporins use and instrumentation or catheterization (Sarma *et al.,* 2011).

In this study, the high risk factors for infection with Multidrug resistance *Klebsiella* isolates were predominantly from patients in Intensive care units-58, followed by other high risk associated factors like previous hospitalisation-42isolates,Diabetes mellitus-37 isolates,surgery /trauma-30 isolates,chr.illness-20 isolates, others including malignancy-13 isolates (Table-4).This is very similar to Sunilkumar Biradar et al (2015) , but Several studies done suggest these as the most common risk factors in patients (Namratha *et al.,* 2015; Abhilash *et al.,* 2010). most common risk factor associated with *Klebsiella* infections was found to be diabetes followed by alcoholism and previous surgeries.

In this study, the most common isolated species was *Klebsiella pneumonia subsp aerogenes* (48%),followed by *Klebsiella oxytoca*(46%),and *Klebsiella pneumonia subsp pneumoniae*(6%). Information about the speciation is limited. However the most commonly isolated species of *Klebsiella* is *Klebsiella pneumoniae subsp aerogenes* as mentioned in Greenwood (18^{th} edition) and Meckie& Mc Cartney& (14^{th} edition). Species identification is critical in the diagnosis and treatment of persons infected with *Klebsiella*. It is also required in disease prevention, patient management, and surveillance of

infection. However, this practice is usually ignored in most of our hospitals mainly due to limited resources, time and labour.

K. pneumonia subsp aerogenes was the most common species isolated (48%), hence responsible for the majority of *Klebsiella* infections and therefore supported the fact that it is the most virulent of all the *Klebsiella* [8] and hence the commonest etiologic agent of both community and hospital acquired infections.

Both Klebsiella species(Klebsiella pneumonia subsp aerogenes, and klebsiella oxytoca) were most commonly isolated in wound infections followed by respiratory infections and urinary tract infections.But *Klebsiella pneumonia subsp pneumoniae* was isolated most commonly in respiratory tract infections followed by others.(Table-6).This is similar to study conducted by Sunilkumar Biradar et al (2015) in which the highest percentage of *Klebsiella* spp were isolated from wound infection(50%) followed by UTI(21%), and RTI(18%). This implies that *Klebsiella* infections commonly affects the patients with breaches in airway and skin integrity due to saphrophytic nature.

The Antimicrobial sensitivity pattern of *Klebsiella* species were studied. In this study, the *Klebsiella pneumoniae* isolates were 67% sensitive to Imipenem, Ertapenem(78%), Piperacillin/tazobactum (51%), Amikacin (80%), , Cefotaxime (18%), Nitrofurantoin (79%), Ceftazidime (18%), Gentamicin (48%), Norfloxacin (79%), Ciprofloxacin (66%), Cotrimoxazole (63%). Klebsiella oxytoca isolates (79%) were sensitive to Imipenem, Ertapenem (77%), Piperacillin / tazobactam (68%), Amikacin (70%), Cefotaxime (11%),

Nitrofurantoin (80%), Ceftazidime (11%), Gentamicin (67%), Norfloxacin (60%), Ciprofloxacin (41%), Cotrimoxazole (65%)(Table-7). The results were interpreted as per CLSI guidelines [9]. In the present study, multidrug resistance was found more common in *K. pneumonia* (56%) than in *K. oxytoca* (50%). In this study it is observed 82%&89% resistance to cefotaxime was exhibited by *Klebsiella pneumoniae* and *K.oxytoca* respectively. It correlates with the study done by Sasirekha et al. (2010) and Singh and Goyal (2003) in India where they found 84% resistance to cefotaxime and 85% resistant to ceftazidime

Hospital acquired isolates were more resistant and it may be due to lack of antibiotic policy, irrational use of 3 generation cephalosporins mainly ceftriaxone in the hospital (Shova 2007) and the emergence of antibiotic-resistant organisms in hospitals in concert with the use of high levels of antibiotics use caused the emergence of resistant organisms and they might be inherently more virulent than the organisms are sensitive (CDC 2002). *Klebsiella spp* were more resistant due to virulence factors like hyperviscosity, polysaccharide capsule and production of endotoxin, carbapenemases (Highsmith and Jarvis1985; Lin et al. 2011). Thomson and associates found *Klebsiella* was more resistant to cefotaxime and ceftazidime (Thomson 2011).

In this study, resistance to Ciprofloxacin was 38% and 50% for *Klebsiella pneumoniae* and *Klebsiella oxytoca*. (Table-V1I) respectively. These findings were low when compared with the study by Haque and Salam (2010) from Bangladesh (90.9%). In another study by Sasirekha from India , 68% strains

were resistant to ciprofloxacin.Aminoglycosides have good activity against clinically important Gram negative bacilli (Gonzalez and Spencer 1998). In the present study ,80% isolates were susceptible to Amikacin, followed by 48% to Gentamicin, it was similar to Sasirekha et al. (2010). Several studies showed that Amikacin was more sensitive than Gentamicin . These variations may be due to increased use of gentamicin, caused by selection pressure of aminoglycosides in different region (Miller and Sabatelli 1997).

Carbapenems are the drugs of choice for many infections caused by Gram positive and Gram negative bacteria (Ullah et al. 2009). In this study ,imipenem was 67% sensitive .These findings were similar to study done by Rashid et al. 2012 in Bangladesh (69%). So, these drug resistance organisms have limited therapeutic options and necessitated the increased use of carbapenems. But recently, new beta lactamases have developed e.g., *K. pneumoniae* carbapenemase (KPC) which is resistant to imipenem and has spread world wide (Rhee et al. 2010). So there is very limited options to treat imipenem resistant strains and colistin may be the drug of choice (Amaya et al. 2009), though it has many side effects.

In the present stuy,*Klebsiella pneumonia*e had higher percentage of resistance to third generation cephalosporins,aminoglycosides, quinolones and carbapenems when compared to *Klebsiella oxytoca* .This is because of multiple virulence factors like production of capsule,biofilm formation,and efflux

pumps.Hence there was a significant difference (p value<0.005)between the antimicrobial sensitivity pattern of *Klebsiella pneumoniae* and other species.

Multidrug resistance among *Klebsiella species* is common because of its potential to respond quickly to the changes in selective environmental pressure .In the present study ,ESBLs prevalence was 59.5% , which was very similar to the study done by Sharma et al. (2010) in India who found 70% ESBLs rate. Studies from India reported ESBL producers as high as 60.98%, 51.4% , 53.4% 67.2% in 2004, 2007 ,2010,and 2014 respectively (Babypadmini 2004; Shivaprakasha 2007; Sasirekha 2010 Guwahati 2014). Higher percentage (66.9%) was given by Gaurav Dalela et al,Sheevani Seemer et al (58%),Bennett JW et al (64.8%)while lower rates of ESBL were reported by Dechen C Tsering et al (34.03%),Meenu Garg et al(41%),Vinothkumar et al(33.33%) . This increasing trend of ESBL producers were due to steadily increasing of ESBL producing strains among the clinical isolates and also it indicate the possibility of treatment failure caused by resistant organisms (Chaudhary 2004; Haque and Salam 2010) . The high occurrence of ESBLs in *Klebsiella spp* is of great concern since infections caused by this bacterium were very common and resistance of the organism may be due to the presence of capsule that gives some level of protection to the cells, presence of multidrug resistance efflux pump, easy spread of organism, efficient at acquiring and disseminating resistance plasmid (Chaudhary and Aggarwal 2004; Gruteke 2003; Yusha.u 2010).

Failure to detect ESBL production by routine disc-diffusion tests has been well documented (Tenover 1999; Paterson 1999). Screening with 3GCs, with clavulinic acid more than one combination increased the rate of ESBL detection, but the combination of ceftazidime and cefotaxime still missed two strains producing the SHV-5 and SHV-7 ESBLs (George et al. 2006). Use of only one combination may fail to detect ESBL positive strains and thus might cause low prevalence (Rahman et al. 2004). The use of Cefotaxime, Ceftazidime and Ceftriaxone as the only indicator of ESBLs screening can no longer be recommended. If only one indicator antibiotic would be used for screening, Cefpodoxime has proven to be the best molecule for screening all types ESBLs producers in clinical sample (Black et al. 2005). Occurrence and distribution of ESBLs differs from country to country and from hospital to hospital (Ali 2009). These types of discrepancies between susceptibility data and disc diffusion results have increased the need for an improved method of ESBL detection and incorporate it into routine susceptibility procedure.

Of the 200 isolates of *Klebsiella species* studied, 48 were resistant to cefoxitin on screening. AmpC disk test was positive in 8 (32%) isolates in *Klebsiella pneumonia* . the prevalence of AmpC 8.4% in K. pneumoniae. The 46 and 48 isolates of *K.pneumoniae* and *K.oxytoca* were resistant to cefoxitin, of this only 13 isolates was AmpC producers . Amp C enzyme has also been described in 3.3 per cent of isolates from Karnataka(117) Cefoxitin resistance in these non producers could be due to some other resistance mechanism such as alterations in outer membrane permeability [81,82]

In the present study, the prevalence of CRE in our hospital is 27.5%. Among carbapenems, 33% isolates of *Klebsiella pneumoniae* and 21%isolates *of Klebsiella oxytoca* were resistant to imipenem. The results of present study were in accordance with Gupta *et al* reported 17-22 per cent of hospital acquired infections among Gram-negative pathogens, while Wattal *et al*-reported 13-51 per cent prevalence of carbapenem resistant *Enterobacteriaceae* (CRE) .The result of present study were low compared to Parveen *et al.* (2010) who reported that 73.3% *Klebsiella* isolates are resistant to imipenem. . Shah et al also reported 86.96% resistance to imipenem (Shah and Desai, 2012). The prevalence of CRE reported from India range from 7-51%[19,22] All the CRE isolates detected by disc diffusion and MIC were also tested by Modified Hodge test. Modified Hodge test is recommened by CLSI as isolates of Enterobacteriaceae producing KPC type carbapenamase have a high level of sensitivity (>90%)and Specificity[7]. In our study out of 55 isolates only 40 isolates are positive for MHT, but 15 isolates are negative,this may be due to overproduction of ESBL or AmpC enzyme with porin loss. As the use of carbapenems to treat infections varies from hospital to hospital, so the level of resistance may vary from hospital to hospital.

The increasing and rapid spread of MBL producing *Enterobacteriaceae*, particularly *E. coli* and *K. pneumoniae* constitutes a serious threat to public health worldwide. The present study indicated a high incidence of MBL producing *K. pneumoniae* (8%) in different clinical samples.this was similar to the study done by Loveena Oberoi et al(2012) it was 10.98% and Bandekar et.al. who reported

15.7% MBL producers. A previous study from an another tertiary care hospital in Nepal reported comparatively lower incidence of MBL producing gram negative bacteria (1.3%) in lower respiratory tract specimens [12]. However, a recent study from Nepal addressed the issue of increasing incidence of MBL producing *K. pneumoniae* (18.2%) in tracheal aspirate samples [13]. Several recent studies from other parts of Asia also demonstrated increasing incidence of MBL production in Enterobacteriaceae isolates[14-16]. In general, production of MBL in Enterobacteriaceae isolates currently follows an increasing prevalence pattern and the prevalence rate may vary greatly in different geographical areas and from institute to institute. In our hospital setting, extended spectrum beta-lactamases are prevalent in *Klebsiella* isolates and there is a gradual rise in the use of carbapenems, which could be a major cause of MBLmediated resistance.

In our study, out of 55 imipenem resistant isolates, only 12 was KPC producers (8%) .This is similar to study done by Christian .F. et.al (5.1%). This is low when compared to the study conducted by Bansal et al (2013) reported 36% KPC producers and Sanjeev kumar et al(2015) reported 36% KPC producers.Out of 16 isolates positive for both MHT and Disc diffusion method only 12 isolates were positive for bla KPC done by Primers designed by HELINI Biomolecules,Chennai.This discrepancy between phenotypic and genotypic may be due to presence of other genes coding for carbapenamases other than KPC.

The coexistence of different classes of β lactamases in a single isolate may pose diagnostic and treatment challanges. In this study, the co production of

ESBL/MBL/KPC/AmpC β lactamases was observed in 25 isolates (12.5%).Out of 25 isolates majority were ESBL+ AmpC -10(5%),followed by MBL+KPC -9(4.5%),AmpC+ MBL-3(1.5%) and ESBL+MBL-3(1.5%).this was similar to study done by Loveena Oberoi et al(2013) which reported the coexistence of ESBL+MBL-8.79% ,AmpC+ESBL-6.59%, AmpC+ MBL-3.67% and Arora et al(2005) reported that AmpC+ MBL-46.6% and ESBL+ AmpC -3.3%. Sanjeev kumar et.al.(2015) reported MBL+KPC -13(26%),Bansal M et al (2014) reported MBL+KPC-14.79%,and Tsakaris A et al(2013) reported MBL+KPC-21.98%.

The treatment of *Klebsiella* infections remain a great challenge because resistant to aminoglycosides,cephalosporins and qinolones has substantially increased worldwide . Carbapenems are the drug of choice for *MDR Klebsiella* infections,for ESBL and AmpC producing isolates,but resistance to carbapenems by the production of carbapenamases and various other mechanisms has limited the therapeutic options.Current options for treatment of CR-Klebsiella isolates include tigecycline and colistin. However, each of these drugs and polymixin B as monotherapy results in frequent failures in treatment compared to combination therapy. Therapeutic options under development against *CR-Klebsiella* isolates are mostly derivatives of polymyxin (*e.g.* NAB739, NAB740); others include β-lactamase inhibitor Clavulanic acid, with combination of oxyimino cephalosporin as well as with ceftazidime.

The clinical outcome of the patients with KPC producing *Klebsiella* infections were determined in this study.Among the 12 patients with KPC

producing *Klebsiella* infections,3 patients expired.The mortality rate in patients with bla KPC gene was 25%.

Hence the prevalence of Klebsiella infections emphasizes the need for early detection of various betalactamases,including carbapenamases,which would help in selection of appropriate antibiotic regimen and prevention of emergence and dissemination of MDR strains.

Summary

SUMMARY

- The present study includes 200 clinically significant,consecutive,non-duplicate *Multidrug resistant Klebsiella isolates.*
- Most of the *Klebsiella* isolates obtained were from pus samples(38%) followed by urine samples(24.5%) followed by sputum (12%),body fluids(9.5%),tracheal aspirates(7%),devices(6%),CSF(1.5%),blood(1.5%).
- Majority of the *multidrug resistant Klebsiella* isolates were from the Intensive care unit followed by other high risk associated factors like previous hospitalisation,diabetes,and surgery/ trauma.
- *Klebsiella pneumoniae subsp aerogenes*(48%) was the most common species isolated followed by *Klebsiella oxytoca*(46%) and *K.pneumoniae subsp pneumonia*(6%).
- There was a significant difference between the antimicrobial sensitivity pattern of *Klebsiella pneumoniae* and *Klebsiella oxytoca* since p value is <0.05 for cephalosporin, aminoglycosides, quinolones, carbapenems and But there was no significant difference for cotrimoxazole.
- 55 isolates (27.5%) were found to be resistant to Imipenem by Kirby Bauer disc diffusion .
- All the 55 isolates have their MIC above 4µg/ml,hence disc diffusion method correlates with MIC.

- Among the 200 isolates of *Klebsiella* species 119(59.5%) were ESBL producers, followed by MBL 16(8%), AmpC 13(6.5%) ,KPC 12(6%) Enzyme coproducers 25(12.5%s, other mechanism 15(7.5%).
- Among the 40 MHT positive isolates ,all the 16 isolates were screened and phenotypically confirmed for *Klebsiella pneumoniae carbapenamase* by EUCAST Guidelines for resistance detection method. Among these isolates only 12 isolates were positive for bla kpc gene.
- The most common Enzyme coproducers ESBL+AMPC were common in *K.p.subsp aerogenes and K.oxytoca* followed by MBL+KPC which is more common in *Klebsiella oxytoca*.

Conclusion

CONCLUSION

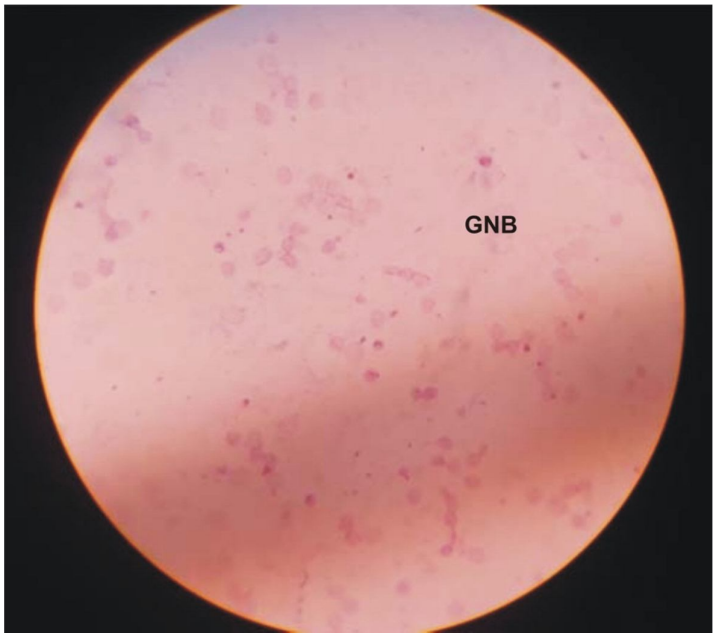
- In this study, 200 Multidrug resistant Klebsiella strains were isolated mainly from Intensive care unit (29%) followed by Orthopedics ward (16.5%). Among these 200 *Multidrug resistant Klebsiella* isolates, *Klebsiella pneumoniae subsp aerogenes (48*%) was the most common species isolated followed by *Klebsiella oxytoca* (46%) and *Klebsiella pneumonia subsp pneumonia* (6%). Among these 200 isolates 119(59.5%) showed ESBL production,16(8%) showed Metallobetalactamases, 13(6.5%)showed AmpC production and 12(6%) showed KPC production and 25(12.5%) were Enzyme co producers. All resistance mechanisms were most common in *Klebsiella pneumonia* than *Klebsiella oxytoca* except Metallobeta lactamases which were more common in *Klebsiella oxytoca*.Though in this study most ESBL & AmpC producers are susceptible to Carbapenems, excessive usage of Carbapenems may lead to the development of its resistance as well as would limits the treatment options.

- In this study, 25(12.5%) isolates showed Enzyme coproduction of ESBL / MBL / KPC / AmpC. The coexistence of different classes of β lactamases in a single isolate may pose diagnostic and therapeutic challenge.

- In this study, MBL and KPC production was in significantly higher which were associated with increased mortality, morbidity and cost.Therefore early detection and routine surveillance of MBL and KPC producing *Klebsiella i*solates is crucial for establishing appropriate empirical antimicrobial therapy and restraining their spread in hospital environment.

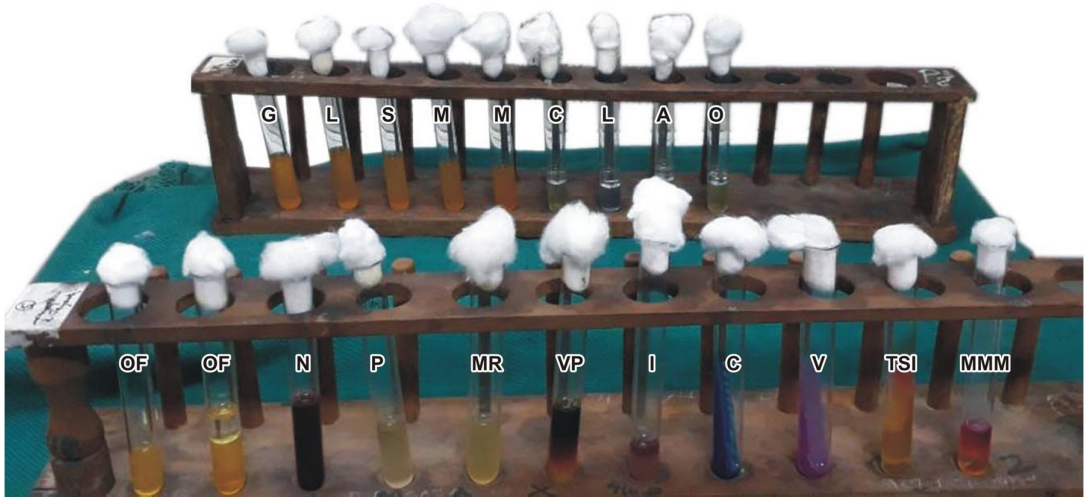
- In view of controlling these resistant strains of *Klebsiella* isolates ,early and simultaneous detection of these resistance pattern (ESBL, AMPC, MBL and KPC) and strict adherence to antibiotic policies by curtailing injudicious use of antibiotics and implementation of antimicrobial Stewardship would prevent the emergence and the cross transmission of multidrug resistant organisms.

Colour Plates



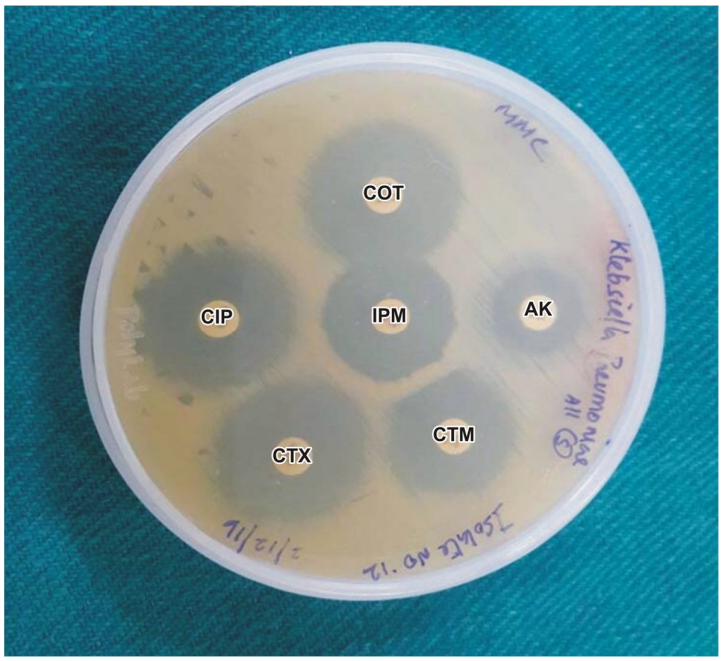
COLOUR PLATE-1:DIRECT GRAM STAINING –CAPSULATED GRAM NEGATIVE BACILLI AND PUS CELLS.

COLOUR PLATE-2:MUCOID LACTOSE FERMENTING COLONIES IN MAC CONKEY AGAR PLATE.



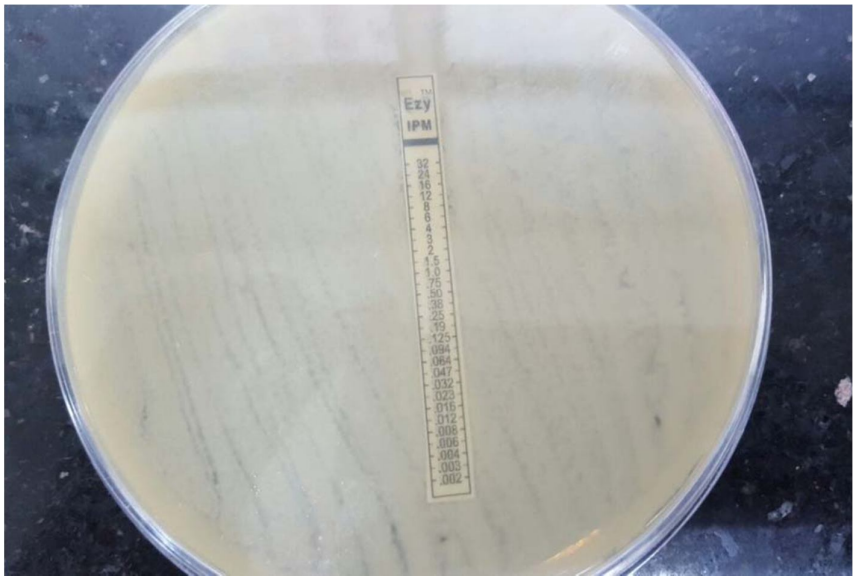
COLOUR PLATE-3:BIOCHEMICAL REACTIONS FOR *Klebsiella Oxytoca.*

COLOUR PLATE-4:BIOCHEMICAL REACTIONS FOR *Klebsiella pneumonia aerogenes.*



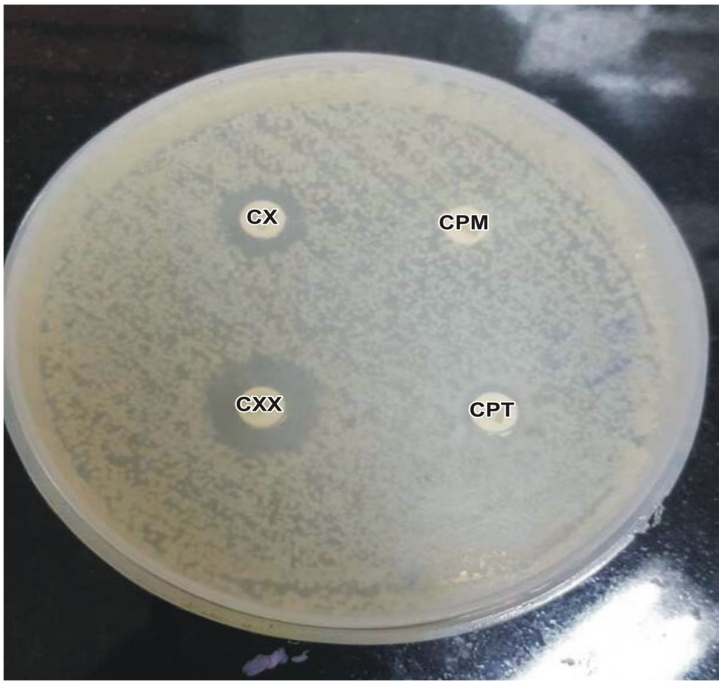
COLOUR PLATE-5: All sensitive *Klebsiella pneumonia subsp pneumoniae* isolate in Muller Hinton Agar plate with Antibiotics.

COLOUR PLATE-6: Imipenem MIC by Epsilometer method-Sensitive strain



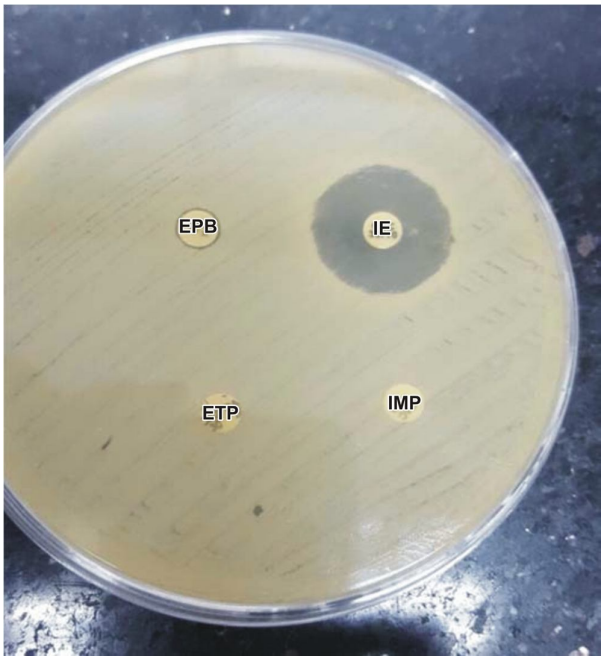
COLOUR PLATE-7: Imipenem MIC by Epsilometer method-Resistant strain.

COLOUR PLATE-8:Extended Spectrum Beta Lactamases(ESBL) producing *Klebsiella pneumoniae* isolate

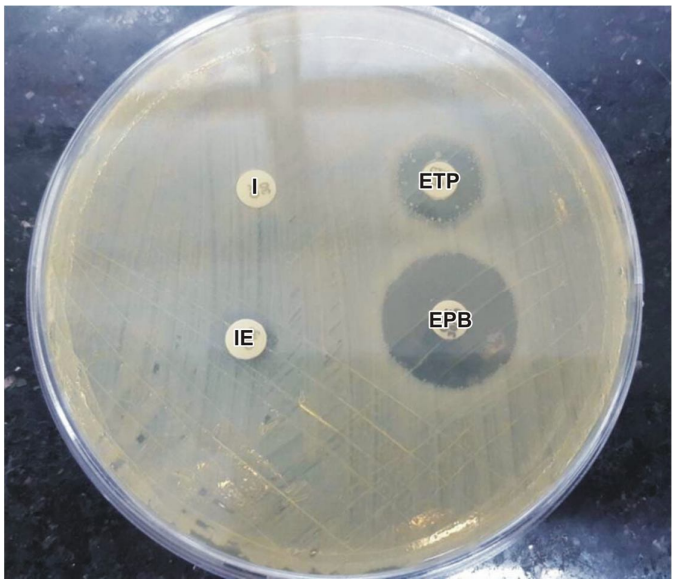


COLOUR PLATE-9:AmpC Beta Lactamases (AmpC) producing *Klebsiella oxytoca* isolate(>5mm)

COLOUR PLATE-10: Modified Hodge Test - Carbapenamases producing *Klebsiella pneumoniae* isolate(CLOVER LEAF APPEARANCE)

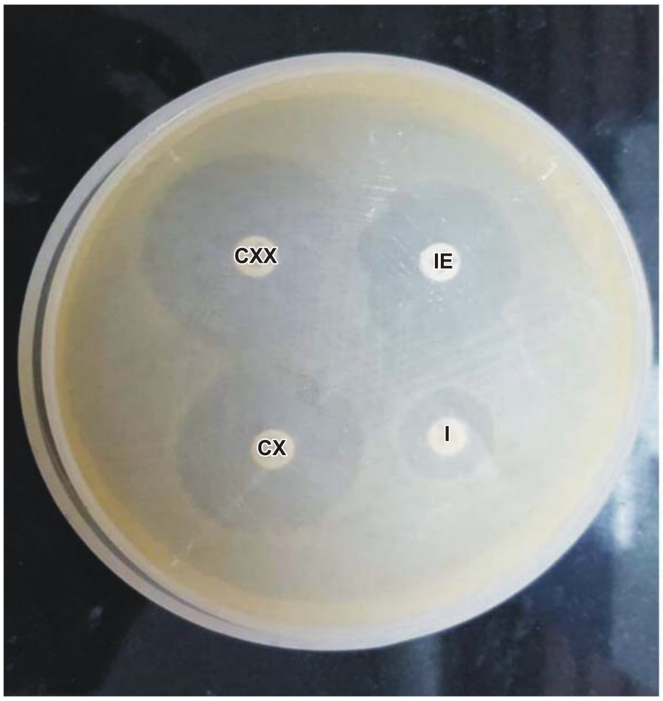


COLOUR PLATE-11:Metallo Beta Lactamases (MBL) producing *Klebsiella pneumoniae* isolate.

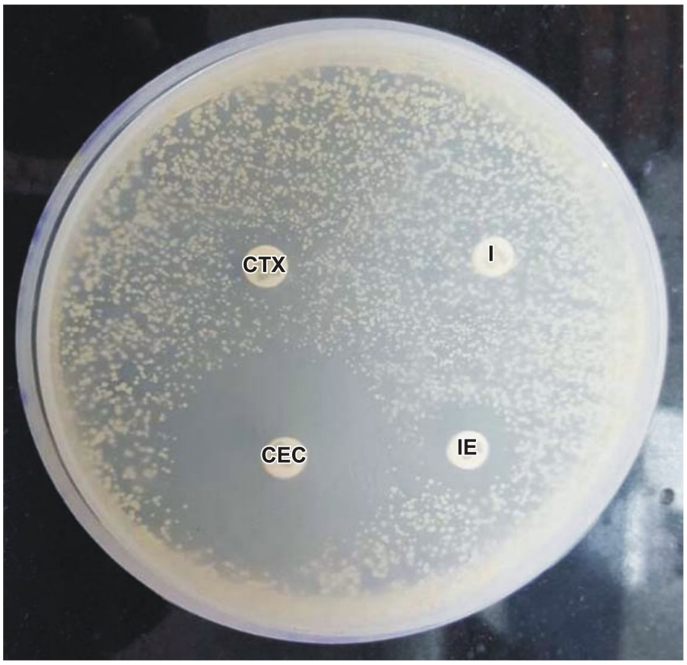


COLOUR PLATE-12:Klebsiella pneumoniae carbapenamases (KPC) producing *Klebsiella oxytoca* isolate(>5mm).

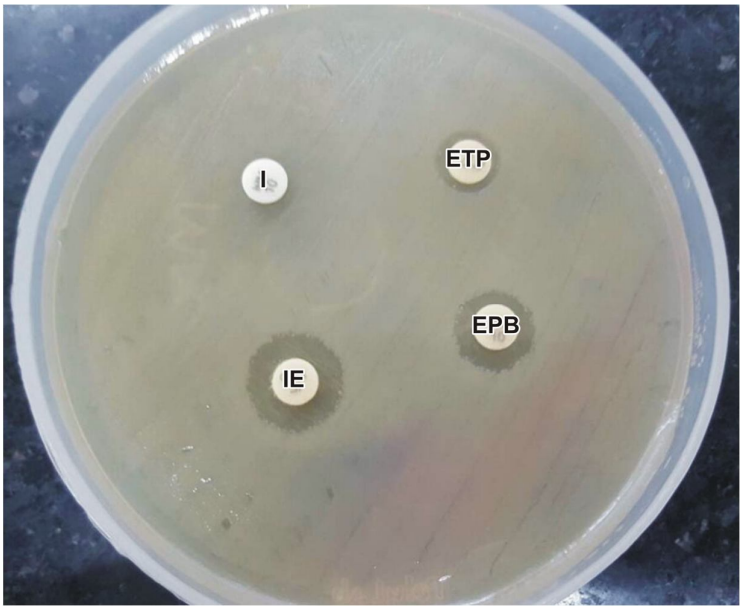
COLOUR PLATE-13:ESBL+AmpC CO- producing *Klebsiella oxytoca* isolate(both >5mm)



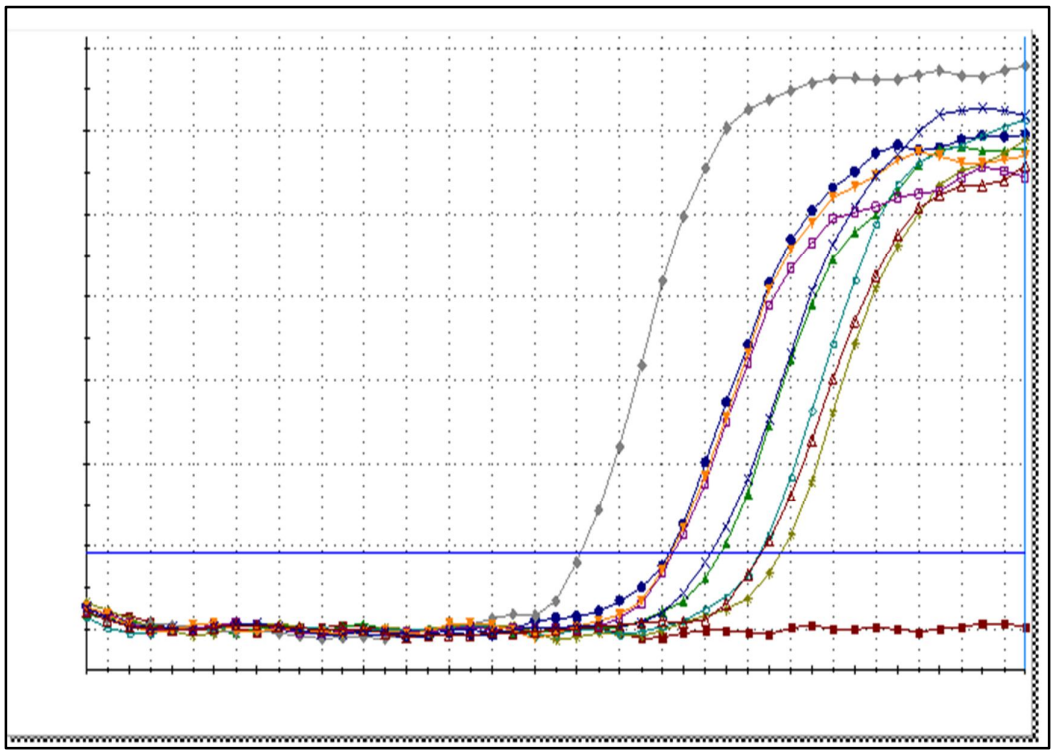
COLOUR PLATE-14:AmpC+MBL CO- producing *Klebsiella pneumonia* isolate(AmpC >5mm&MBL>7mm).



COLOUR PLATE-15:ESBL+MBL CO- producing *Klebsiella oxytoca* isolate ESBL >5mm&MBL>7mm).



COLOUR PLATE-16:MBL+KPC CO- producing *Klebsiella oxytoca* isolate(KPC >5mm&MBL>7mm).



COLOUR PLATE-17:RT PCR –FOR bla KPC GENE

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APPENDIX-1

ABBREVIATIONS

AmpC	-	Ampicillin resistant gene
ATCC	-	American Type Culture Collection
BA	-	Blood AgaR
bp	-	Base pairs
BSAC	-	British Society for AntimRicrobial Chemotherapy
CA	-	Clavulinic acid
CDC	-	Center for Disease Control and Prevention
Cfu	-	Colony forming unit
CLSI	-	Clinical laboratory standard institute.
CPM	-	Cefipime
CSF	-	Cerebrospinal fluid
CRE	-	carbapenem-resistant *Enterobacteriaceae*
TX-M	-	Active on Cefotaxime, First Isolated at Munich . Cefotaxime resistant gene
CWT	-	Cell wall transamidase
DDM	-	Disc diffusion method
DDST	-	Double disc synergy test
DNA	-	Deoxyribonucleic acid
dNTP	-	Deoxynucleoside triphosphate
E test	-	Epsilon test(Epsilometer)
EDTA	-	Ethylene diamine tetraacetic acid

ESBL-KP	-	ESBL producing *K. pneumonia*
et al	-	at alia (all others)
EUCAST	-	European Committee on Antimicrobial Susceptibility Testing
GNR	-	Gram negative rods
HGT	-	Horizontal gene transfer
IBCs	-	Intracellular bacterial communities
kbp	-	Kilo base pair
KPC	-	Klebsiella pneumoniae Carbapenemase
LPS	-	lipopolysaccharide
MA	-	MacConkey agar
MBL	-	Metallo beta lactamase
MDR	-	Multidrug resistant
MHA	-	Mueller Hinton Agar
MIC	-	Minimum inhibitory concentration
MRSA	-	Methicillin resistant *Staphylococcus aureus*
MYSTIC	-	Meropenem Yearly Susceptibility Test Information Collection
NCBI	-	National Center for Biotechnology Information
NA	-	Nutrient agar
NAG	-	N-acetylglucosamine
NAM	-	N-acetylmuramic
NCCLS	-	National Committee for Clinical Laboratory Standards
OM	-	Outer membrane

OXA	-	Oxacillinase gene
PBP	-	Penicillin binding protein
PBS	-	Phosphate buffer saline
PCR	-	Polymerase chain reaction

Antibiotics

CTX	-	Cefotaxime
CTR	-	Ceftriaxone
CAZ	-	Ceftazidime
CAC	-	Ceftazidime + clavulanic acid
CEC	-	Cefotaxime+clavulanic acid
CN	-	Cefoxitin
CPM	-	Cefepime
CFC	-	Cefepime+clavulanic acid
CPT	-	Cefepime+tazobactam
CMC	-	cefixime+clavulanic acid
CA	-	clavulanic acid
IMP	-	Imipenem
ERT	-	Ertapenem
AK	-	Amikacin
GM	-	Gentamycin
COT	-	Trimethoprim - Sulfamethaxozole

APPENDIX-II

A).STAINS AND REAGENTS

Gram staining:

- Methyl violet(2%)-10g of Methyl violet in 100 ml Absolute alcohol in 1 litre of Distilled water.(primary stain)
- Grams Iodine-10g Iodine in 20 g KI(fixative)
- Acetone-Decolourizing agent.
- Carbol fuchsin(1%)-Secondary stain.

MEDIA USED

MacConkey agar medium

Composition

Ingredients gram/liter

- Peptone 20g
- Lactose 10g
- NaCl 5.g
- Na- Deoxycholate 1.0
- Neutral Red 0.03
- Agar 15.0

Fifty-two grams of dehydrated MacConkey agar medium was suspended in 1000 ml cold distilled water and boiled to dissolve the medium completely. The solution was then sterilized by autoclaving at 121°C and 15 lbs pressure for 15 minutes.

Blood agar medium(5% sheep blood agar)

Composition

Ingredients	**gram/liter**
Peptone	10.00
Distilled water	1 ltr.
Sodium chloride	5.00
Agar	15.00

Forty grams of the dehydrated blood agar medium was suspended in 1000 ml cold distilled water in a flask and boiled to dissolve the medium completely. It was then sterilized by autoclaving at 1210C and 15 lbs pressure for 15 minutes. The autoclaved materials were allowed to cool to a temperature of 450C in a water bath. Defibrinated 5-10% sheep blood was then added to the medium aseptically and distributed to sterile petridishes. Sterile media was stored in refrigerator at 40C for future use.

Muller Hinton agar medium

Composition

Ingredients	**gram/liter**
Beef dehytrated infusion	300
Casein hydrolysate	17.50
Starch agar	1.50
Agar	10.00

Thirty-eight grams of dehydrated Mueller Hinton agar medium was suspended in 1000 ml cold distilled water and boiled to dissolve the medium completely. The solution was then sterilized by autoclaving at 121°C and 15 lbs pressure for 15 minutes. The autoclaved media was stored at 4°C.pH=7.4

MEDIA REQUIRED FOR BIOCHEMICAL IDENTIFICATION:

1.Catalase test:3% hydrogen peroxide

Oxidase reagent

Composition

Distilled water 10ml

Tetramethyl-P- phenylenedimine 0.1 g

Indole test

Composition

Ingredients amount

Peptone	20g
Sodium chloride	5g
Distilled water	1 L

After adjustment of the pH to 7.4 , sterilize by autoclaving at 1210C for 15 min.

Kovac.s reagent

Amyl or isoamyle alcohol	150ml
p . Dimethyl-aminobenzaldehyde	10g
Hydrochloric acid	50ml

Dissolve the aldehyde in the alcohol and slowly add the acid and store in the refrigerator.

Simmon's Citrate Medium:

- Koser's medium 1 ltr
- Agar 20g
- Bromothymol blue 0.2% 40ml
- Dispense,Autoclave at 121°for 15 min and allow to set as slopes.

Triple Sugar Iron medium:

- Beef extract 3g
- Yeast extract 3g
- Peptone 20g
- Glucose 1g
- Lactose 10g
- Sucrose 10g
- Ferric citrate 0.3g
- Sodium chloride 5g
- Sodium thiosulphate 0.3g
- Agar 12g
- Phenol red 0.2% solution 12 ml
- Distilled water 1 ltr

Heat to dissolve the solids,add the indicator solution,mix and tube .Sterilize at 121° for 15 min and cool to form slopes with deep butts.

Methyl Red test/Voges –Proskauer test:

A.MR/VP broth(Glucose broth/phosphate buffer broth)

Polypeptone	7g
Glucose	5g
Dipotassium phosphate	5g
Distilled water	1Ltr
Final pH	6.9

B.Reagents

1.∝-Napthol,5%(5gm in 100ml of absolute ethyl alcohol)

2.Potassium hydroxide 40%(Potassium hydroxide in 100ml of Distilled water).

Decarboxylase media:

Moller decarboxylase broth base:

Ingredients	gms/ml
Peptone	5
Beef extract	5
Bromocresol purple	0.01
Cresol red	0.005
Glucose	0.5
Pyridoxal	0.005

Aminoacid

Add 10g of the levo form of the aminoacid for 1000 ml.mix and dispense in sterile tubes.

Hugh-Leifson's Oxidation-Fermentation test:

Peptone	2g
Sodium chloride	5g
D-glucose	10g
Bromothymol blue	0.03g
Agar	3g
Dipotassium phosphate	0.3g
Distilled water	1ltr

pH=7.1

Basal medium is autoclaved.1%of sterile sugar solutions is added to the basal medium.Dispense into the sterile test tubes without slant.

EDTA Solution:

0.5M EDTA solution was prepared by adding 186.1 gm of disodium EDTA in 1000 ml of distilled water.pH was 8.Sterilized by autoclaving at 121°C for 15 min.

PhenylBoronic Acid stock solution:

20 mg of Phenyl boronic acid(benzene boronicacid:Himedia)in Dimethyl sulfoxide at a concentration of 20mg/ml,from this 20µl(400µg of boronic acid)was dispensed to Ertapenem disc.

McFarland Standard 0.5

Composition and preparation 1 % (V/V) solution of chemically pure (0.36N) Sulphuric acid and 1.175 % (W/V) solution of chemically pure (0.048M) barium chloride was prepared in two separate sterile flasks. Then 9.9 ml of sulphuric acid and o.1 ml of barium chloride were added to the clean screw capped test tube and sealed. The barium sulphate suspension corresponds approximately to McFarland standard tube No.1 with corresponding cell density of 3 × 108 organisms/ml. To made the turbidity standard of cell density to one half of the McFarland standard tube No.1 which correspond to cell density of 1.5 × 108 organism/ml for determination of antibiotic sensitivity by Kirby-Bauer inoculated technique 0.5 ml of 1.7 %(W/V) barium chloride (Bacl2 2H2O) was added to 99.5 ml of 1 % (V/V) Sulphuric acid (0.36N), mixed well and 5- 10 ml was distributed in sterile capped test tubes and sealed.

ANNEXURE – I

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI 600 003

EC Reg.No.ECR/270/Inst./TN/2013
Telephone No.044 25305301
Fax: 011 25363970

CERTIFICATE OF APPROVAL

To
Dr.M.Sathiya
Post Graduate in M.D. (Microbiology)
Institute of Microbiology
Madras Medical College
Chennai 600 003

Dear Dr.M.Sathiya,

The Institutional Ethics Committee has considered your request and approved your study titled "**DETECTION OF MULTIDRUG RESISTANCE IN KLEBSIELLA SPECIES BY PHENOTYPIC AND GENOTYPIC METHOD IN A TERTIARY CARE HOSPITAL" - NO.(II) 17032016.**

The following members of Ethics Committee were present in the meeting hold on **22.03.2016** conducted at Madras Medical College, Chennai 3

1.Dr.C.Rajendran, MD.,	:Chairperson
2.Dr.R.Vimala,MD.,Dean,MMC,Ch-3	:Deputy Chairperson
3.Prof.Sudha Seshayyan,MD., Vice Principal,MMC,Ch-3	: Member Secretary
4.Prof.P.Raghumani,MS, Dept.of Surgery,RGGGH,Ch-3	: Member
5.Dr.Baby Vasumathi, Director, Inst. of O&G,Ch-8	: Member
6.Prof.M.Saraswathi,MD.,Director, Inst.of Path,MMC,Ch-3	: Member
7.Prof.Srinivasagalu,Director,Inst.of Int.Med.,MMC,Ch-3	: Member
8.Tmt.J.Rajalakshmi, JAO,MMC, Ch-3	: Lay Person
9.Thiru S.Govindasamy, BA.,BL,High Court,Chennai	: Lawyer
10.Tmt.Arnold Saulina, MA.,MSW.,	:Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

MADRAS MEDICAL COLLEGE
31 MAR 2016
CHENNAI-600 003

Member Secretary – Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

ANNEXURE-II

PROFORMA

- Name : IP NO:
- Age: Ward:
- Sex:
- Occupation:
- Address:

- Presenting complaints

- Personal history

- Past history

- Prior antibiotic therapy

- Microbiological investigation:

- Direct examination -

- Culture :bacterial culture

- Speciation.

    - Antibiotic sensitivity pattern:-

PCR for KPC bla gene:

ANNEXURE-III

CONSENT FORM

STUDY TITLE : Detection of Multidrug Resistance in Klebsiella by Phenotype and Genotype Methods in a Tertiary Care Hospital

I……, hereby give consent to participate in the study conducted by Dr.M.Sathiya, Post graduate at Institute of Microbiology, Madras Medical College, Chennai and to use my personal clinical data and the result of investigations for the purpose of analysis and to study the nature of the disease, I also give consent to give my clinical Specimen (sputum, endotracheal aspirate, bronchial wash, urine, pus, blood) for further investigations. I also learn that there is no additional risk in this study. I also give my consent for my investigator to publish the data in any forum or journal.

Signature/ Thumb impression Place Date

Of the patient/ relative

Patient Name & Address:

Signature of the investigator:

Signature of guide

சுய ஒப்புதல் படிவம்

ஆய்வு செய்யப்படும் தலைப்பு :

மூன்றாம் நிலை மருத்துவமனையில் பல்வேறு ஆண்டிபயாடிக் மருந்துகளுக்கு எதிர்ப்பு தன்மை உடைய கிளப்ஃசெல்லா (Klebsiella) என்னும் கிருமிகளின் நோய் தாக்கம் விகிதம் மற்றும் மருந்துகளின் எதிர்ப்பு தன்மைக்கு காரணமான மூலக்கூறு பாத்திரப்படைப்பை RT-PCR மூலம் கண்டறியும் ஆய்வு.

பெயர் : வயது : தேதி : பங்கேற்பாளர் எண் :

..... என்பவராகிய நான் இந்த ஆய்வின் விவரங்களும் அதன் நோக்கங்களும் முறையாக மருத்துவரிடம் கேட்டு அறிந்து கொண்டேன். எனது சந்தேகங்கள் அனைத்திற்கும் தகுந்த விளக்கம் அளிக்கப்பட்டது. இந்த ஆய்வில் முழு சுதந்திரத்துடன் மற்றும் சுயநினைவுடன் பங்கு கொள்ள சம்மதிக்கிறேன்.

எனக்கு விளக்கப்பட்ட விஷயங்களை நான் புரிந்து கொண்டு நான் எனது சம்மதத்தைத் தெரிவிக்கிறேன். இச்சுய ஒப்புதல் படிவத்தை பற்றி எனக்கு விளக்கப்பட்டது.

இந்த ஆய்வினை பற்றிய அனைத்து தகவல்களும் எனக்கு தெரிவிக்கப்பட்டது. இந்த ஆய்வில் எனது உரிமை மற்றும் பங்கினை பற்றி அறிந்து கொண்டேன்.

இந்த ஆய்வில் பிறரின் நிர்பந்தமின்றி என் சொந்த விருப்பத்தின் பேரில் நான் பங்கு பெறுகிறேன். இந்த ஆராய்ச்சியில் இருந்து நான் எந்நேரமும் பின் வாங்கலாம் என்பதையும் அதனால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் நான் புரிந்து கொண்டேன்.

இந்த ஆய்வில் கலந்து கொள்வதன் மூலம் என்னிடம் பெறப்படும் தகவலை ஆய்வாளர் இன்ஸ்டிட்யூசனல் எத்திக்ஸ் கமிட்டியினரிடமோ, அரசு நிறுவனத்திடமோ தேவைப்பட்டால் பகிர்ந்து கொள்ளலாம் என சம்மதிக்கிறேன்.

இந்த ஆய்வில் முடிவுகளை வெளியிடும்போது எனது பெயரோ, அடையாளமோ வெளியிடப்பட்டாது என அறிந்து கொண்டேன். இந்த ஆய்வின் விவரங்களைக் கொண்ட தகவல் தாளைப் பெற்று கொண்டேன். இந்த ஆய்விற்காக சேகரிக்கப்படும் மாதிரிகளில் (சளி, சிறுநீர், இரத்தம், நுரையீரல் சேர்ந்துள்ள நீர், மூச்சுக்குழாயில் இருந்து எடுக்கப்படும் திரவம்) இருந்து பரிசோதனை செய்துக் கொள்ள சம்மதிக்கிறேன்.

இந்த ஆய்வில் பங்கேற்கும் பொழுது ஏதேனும் சந்தேகம் ஏற்பட்டால், உடனே ஆய்வாளரை தொடர்பு கொள்ள வேண்டும் என அறிந்து கொண்டேன்.

இச்சுய ஒப்புதல் படிவத்தில் கையெழுத்திடுவதன் மூலம் இதிலுள்ள அனைத்து விஷயங்களும் எனக்கு தெளிவாக விளக்கப்பட்டது என்றும் தெரிவிக்கிறேன். இச்சுய ஒப்புதல் படிவத்தின் ஒரு நகல் எனக்கு கொடுக்கப்படும் என்றும் தெரிந்து கொண்டேன்.

பங்கேற்பாளர் கையொப்பம்

தேதி :

ஆய்வாளர் கையொப்பம்

தேதி :

ANNEXURE IV - MASTER CHART

S. No.	Age / Sex	Ward No / IP OP No.	WARD	Diagnosis	Specimen	Organism Identified	Species	CTX	CAZ	CX	AK	GM	CIP	COT	PT	IMP	MHT	Resistant Pattern	Gene Identified
1	24Y/M	223/65609	SUR	Intraperitoneal abscess	Aspirated fluid	Klebsiella	oxytoca	R	R	R	S	S	R	R	R	S		E	
2	45Y/M	30 'S/47187	SUR	lt gluteal abscess	pus	Klebsiella	oxytoca	R	R	R	R	R	R	R	R	R		E+M	
3	24Y/F	234/65599	ORTHO	RTA/hip disarticulation.	wound discharge	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	R	R	R	S		E	
4	50Y/M	29,OR-3/ 64519	ORTHO	post ilizarov tech/wound infection .	pus	Klebsiella	pneumoniae subsp pneumoniae	R	R	R	S	S	R	R	R	S		E	
5	38Y/M	142/67341	SGE	chr.calcific pancreatitis/CKD/DM	sputum	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	S	S	S	S		E	
6	49Y/M	47/63979	URO	BPH/uretheral stent done	urine	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	R	S	R	R	R	P	M+K	Negative
7	66Y/M	215/64167	NEURO	SHT/CVA-LT hemiplegia	urine	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	S	S	R	R	S		E	
8	48Y/F	122/64220	RT	Ca Cervix-under RT/UTI	urine	Klebsiella	oxytoca	R	R	R	S	S	R	R	R	R		R	
9	42Y/M	47/85403	URO	Post renal transplant	pus	Klebsiella	pneumoniae subsp pneumoniae	R	R	R	S	S	R	R	R	S		E	
10	66Y/F	49/64051	RT	Ca Cervix-under RT/UTI	urine	Klebsiella	oxytoca	R	R	R	R	R	R	R	R	R	P	M+K	Negative
11	59Y/F	234/63404	ORTHO	Accidental fall/pelvic injury	sputum	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	S	S	S	S	S		E	
12	20Y/M	142/44650	DM-2	Type 1 DM/IDDM?ulcer RT foot	pus	Klebsiella	oxytoca	R	R	R	R	S	R	R	R	R		E+M	
13	49Y/F	124/63657	DM-1	DM/CKD/UTI	DRAIN	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	S	R	R	R	R		E+M	
14	19Y/M	N4N7/62268	NEURO	RTA/semiconscious state .LT SDH/VAP	sputum	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	S	R	R	R	S		E+A	
15	62Y/F	53/TM-1/44734	TM-1	DM/non resolving pneumoniae.	Bronchial wash.	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	R	R	R	R	P	K	
16	29Y/M	47/62563	NEPHRO	severe alcoholic /CKD/PD dialysis	Peritoneal fluid	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	S	S	S	S	S		E	
17	25Y/M	124/65572	MED-3	RVD/HBsAG+/Pul.Tb	Sputum	Klebsiella	oxytoca	R	R	R	R	S	R	R	R	R		R	
18	52Y/M	121/39995	DM-2	Diabetic foot synd/CVA	pus	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	S	R	R	R	S		E	
19	62Y/M	124/DM-3/64594	TM-2	OLD PUL.TB/DM/URI.	sputum	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	S	S	S	S	S	P	E+A	
20	35Y/M	212 IMCU/93925	IMCU	Sepsis/SHT/ARDS	BLOOD	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	S	R	R	R	S		E	

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21	43Y/M	205/46560	IMCU	PUO/AFI/THROMBOCYTOPENIA/?DHF	Sputum	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	R	S	S	S	S		E	
22	27Y/M	221/96650	MED-3	Sepsis/SHT/CVD/It.Hemiplegia./Bed sore	wound discharge/pus	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	S	S	S	S	S		E	
23	40Y/M	121/54881	MED-1	SHT/Sepsis/CVA	BLOOD	Klebsiella	oxytoca	R	R	R	R	S	S	S	S	S		R	
24	59Y/F	231/55041	DM-1	Lt Diabetic foot synd/AK amputation	wound pus	Klebsiella	oxytoca	R	R	R	S	S	R	R	R	R	P	K	PRESENT
25	24Y/M	NS-1/39417	NEUROSUR-ICU	Post meningitis hydrocephalus/RT VP shunt.	CSF	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	S	S	S	S	S		E	
26	60Y/M	25/67562	TM-2	OLD PUL.TB/DM/LRI/acute exaberationof pneumonia	sputum	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	S	R	R	R	S		E	
27	20Y/M	212/70474	IMCU	AGE/Severe Dehydration/2˚hypovolemia	urine	Klebsiella	oxytoca	S	S	S	R	S	S	S	S	S		A	
28	50Y/M	212/71314	NEURO-ICU	CVA/Aspiration pneumoniae	Tracheal aspirates	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	S	R	R	R	S		E	
29	36Y/M	215/77697	NEURO-ICU	fungal sinusitis/Cavernous sinus thrombosis	PUS	Klebsiella	pneumoniae subsp aerogenes	S	S	S	R	R	S	S	S	S		A	
30	48Y/M	TM-2 /96697	TM-2	COPD/Acute exaberation	sputum	Klebsiella	pneumoniae subsp pneumoniae	R	R	R	R	R	S	S	S	S		E	
31	20Y/F	212/85253	IMCU	Post LSCS/?Sepsis	Ascitic fluid	Klebsiella	oxytoca	R	S	S	R	R	R	R	R	S		E+A	
32	50y/F	125/67402	MED	SHT/LRI	sputum	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	S	R	R	R	S		E	
33	25y/m	214/54123	ORTHO	RTA/Lt BB #Leg/ORIF	wound swab	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	R	R	R	S		E	
34	35y/F	20/63019	ORTHO	D12l1 Disc prolapse/post stabilisation wound .	pus	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	R	R	R	R	P	K	PRESENT
35	37y/M	32/55654	ORTHO	RTA/Lt BB #Leg/ORIF	pus	Klebsiella	pneumoniae subsp pneumoniae	R	R	R	S	S	R	R	R	S		E	
36	20y/F	133/56605	SUR	It.TB Lymphadenitis neck.	pus	Klebsiella	oxytoca	R	R	R	S	R	S	S	S	S		E	
37	39y/M	244/30127	NEUROSUR-ICU	Lt wedge compression ,post stabilisation.	pus	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	R	S	S	S	S		E	
38	50y/M	254/47645	SGE-ISCU	Periampullarey CA.of pancreas/whipple's procedureddone	DRAIN	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	R	S	S	S	S	P	K	PRESENT
39	22y/M	N3N4/67500	NEUROSUR	Post meningitis hydrocephalus/RT VP shunt.	tracheal aspirate	Klebsiella	oxytoca	R	R	R	R	S	S	S	S	S	P	K	PRESENT
40	60y/M	47/51378	URO	BPH/uretheral stent done	urine	Klebsiella	pneumoniae subsp aerogenes	S	S	S	R	R	R	R	R	S		A	
41	35y/M	201/64513	NEUROSUR-ICU	Accidental fall/headinjury/SDH/Drilling	treacheal aspirates	Klebsiella	oxytoca	S	S	S	S	R	R	R	R	S		A	

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42	35Y/M	22/71309	ORTHO	post WDCPfor shaft of femur.	pus	Klebsiella	oxytoca	R	R	R	R	S	R	R	R	S		E	
43	48y/F	233/45740	SUR	Incisional hernia/post op SSI	pus	Klebsiella	pneumoniae subsp pneumoniae	S	S	S	S	R	R	R	R	S		A	
44	39y/M	124/67841	MED	COPD/Acute exaberation	sputum	Klebsiella	pneumoniae subsp pneumoniae	R	R	R	S	R	R	R	R	S		E	
45	30y/M	25/63850	TM-1	Chr.bronchitis	Bronchial wash.	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	S	R	R	R	S		E	
46	48Y/M	124/63850	MED	PTB sequlae/UTI	urine	Klebsiella	pneumoniae subsp pneumoniae	R	S	S	R	R	S	S	S	S		E+A	
47	56y/M	124/63657	URO	CKD/UTI	urine	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	R	S	S	S	S		E	
48	34Y/M	29/57095	SUR	Lt Inguinal hernia/post hernia repair-SSI	pus	Klebsiella	oxytoca	R	R	R	S	R	S	S	S	S		E	
49	33y/M	224/85861	VS-2	RTA/#BBLT.Leg.Raw area rt foot	wound swab	Klebsiella	oxytoca	R	R	R	S	R	S	S	S	S		E	
50	25y/F	245/52316	SGE-ISCU	Pancreatic pseudocyst/abscess.	DRAIN	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	R	R	R	R	R		R	
51	24y/M	167/140327	PWD-ortho 2	RT.Supra condylar femur#	pus	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	R	S	S	S	S		E	
52	20Y/M	244/35642	ORTHO-ISCU	D6#.post stabilisation	DRAIN TUBE	Klebsiella	oxytoca	R	R	R	R	R	R	R	R	R	P	M	
53	60y/M	47/65054	URO-III	BPH/UTI	urine	Klebsiella	pneumoniae subsp pneumoniae	R	R	R	R	R	R	R	S	S		E+A	
54	71/M	49/64987	URO-III-ICU	Autonomic neuropathy on catheter	urine	Klebsiella	oxytoca	R	R	R	R	R	R	R	R	R	p	A+M	
55	35/M	124/65592	ART	RVD/HbsAG+/UTI	urine	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	S	R	R	S	S		E	
56	36/M	111/64135	TM-II	COPD/Acute exaberation	sputum	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	R	R	R	R	R		R	
57	27/M	47/68013	TMI	LRI/Acute exacerbation	sputum	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	R	R	R	R	R		R	
58	38/F	124/59889	TM-II	COPD/Acute exaberation	Bronchial wash.	Klebsiella	oxytoca	S	S	S	S	S	R	R	S	S		A	
59	50/M	N10/57641	N5	Cerebrovascular accident/COMA stage	Tracheal aspirate C/S	Klebsiella	pneumoniae subsp aerogenes	S	S	S	R	R	R	R	S	S		A	
60	40/M	500/58067	ORTHO-I	RTA/forefoot amputation	wound swab	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	R	R	S	S		E	
61	60/M	243/51362	SGE-1	Periampullarey CA.of pancreas/whipple's proceduredone	Bile	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	R	R	R	S	S		E	
62	38/M	43/28273	ORTHO- II	Fracture patella Rt/ Traumatic fracture of 2nd and 3rd metatarsal bone-ORIF done	pus	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	R	R	R	S	S		E	

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63	45/M	30/54856	DM-I	DFS lt foot/Amputation done	pus	Klebsiella	oxytoca	R	R	R	R	R	S	S	R	R	P	K	PRESENT
64	40/F	NSN4/60152	NS-IV	Lt FTP decompressive craniotomy done for RTA/Lt epidural haemetoma	wound swab	Klebsiella	oxytoca	R	R	R	S	R	S	S	R	R	P	M+K	
65	20Y/F	212-58176	IMCU	AFI with thrombocytopenia/?Dengue haemorrhagic fever	sputum	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	S	S	S	S		E	
66	39/M	145/51428	TM-II	COPD/Acute exaberation	sputum	Klebsiella	pneumoniae subsp pneumoniae	R	R	R	S	S	R	R	S	S	P	E+A	
67	40/M	49/44503	URO-III	CKD/Post renal transplant/UTI	urine	Klebsiella	oxytoca	R	R	R	S	S	R	R	R	R	P	M+K	
68	28/F	25/50372	MED	G2P2L1A0/2nd trimesteer	urine	Klebsiella	oxytoca	S	S	S	R	R	S	S	S	S		A	
69	50/M	223/59891	SUR-II	Fournier's Gangrene penis	wound c/s	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	R	S	S	S	S		E	
70	46/M	43/57956	ORTHO-III	GR II distal lt side fracture/compund 5th metatarsal fracture lt foot	pus	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	S	S	S	S		E	
71	55y/M	27/58368	ORTHO-11	Soft tissue sarcoma lt thigh	pus	Klebsiella	oxytoca	R	R	R	S	S	S	S	S	S	P	E+M	
72	40y/F	29/52724	SUR	DFS lt foot/Amputation done	pus	Klebsiella	pneumoniae subsp pneumoniae	R	R	R	S	S	R	R	S	S		E+A	
73	40y/M	29/52724	ORTHO-11	RTA/compound # of RT Patella	pus	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	R	R	R	S	S		E	
74	20y/F	49/42090	ENT-II	LT.CSOM/CENTRAL PERFORATION	AURAL SWAB	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	S	S	S	S	S		E	
75	60Y/M	241/61645	GERIATRIC MEDICINE-ICU	SHT/CVA-LT hemiplegiaBED SORE	PUS	Klebsiella	oxytoca	R	R	R	R	R	S	S	S	S		E	
76	48Y/M	21/60648	ORTHO-III	ACCIDENTAL FALL/COMPOUND #PPX K'WIRE FIXATION	PUS	Klebsiella	oxytoca	R	R	R	R	R	S	S	S	S		E	
77	60Y/M	19/25941	TM-II	COPD/Acute exaberation	sputum	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	S	S	S	S		E	
78	38Y/M	206/61005	TM-1	COPD/Acute exaberation	SPUTUM	Klebsiella	pneumoniae subsp aerogenes	S	S	S	S	R	S	S	S	S		A	
79	48Y/M	143/61060	MED-II	SHT/AGE/SEVERE DEHYDRATION/URI	SPUTUM	Klebsiella	pneumoniae subsp pneumoniae	S	S	S	S	S	S	S	S	S		A	
80	34Y/M	29/60534	ORTHO-III	RTA/CRUSH INJURY RT LEG	PUS	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	S	S	S	S		E	
81	40Y/F	225/61397	SUR-III	INGUINAL HERNIA LT/LAPROTOMY MESH REPAIR	PUS	Klebsiella	oxytoca	R	R	R	S	S	S	S	S	S	P	E+M	
82	34Y/F	121/57072	MED-I	GBS/ASC PARALYSIS LL /BED SORE	PUS	Klebsiella	oxytoca	R	R	R	R	R	S	S	S	S		E	
83	33Y/F	226/26994	NEURO-2	MYELITIS/LT LEG ULCER	WOUND SWAB	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	R	R	R	S	S		E	

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84	47Y/M	214/60491	TOXICOLOGY-ICU	OPC POISONING/SEPSIS	URINE	Klebsiella	pneumoniae subsp pneumoniae	R	R	R	S	S	R	R	S	S		E	
85	28Y/F	212/50372	IMCU	POST LSCS/SEPSIS	URINE	Klebsiella	oxytoca	R	R	R	R	R	R	R	R	R		R	
86	20Y/M	NS-IV/52311	NEUROSUR-ICU	RTA/HEAD INJURY/EXCISION OF EDH	TRACHEAL ASPIRATE	Klebsiella	oxytoca	R	R	R	S	S	S	S	R	R	P	M+K	
87	35Y/M	248/60202	SGE-II	STOMACH CA.CORECTIVE SURGERYDONE	PUS	Klebsiella	oxytoca	R	R	R	R	R	R	R	S	S		E	
88	6OY/M	41/51032	VS-2	DFS lt foot/Amputation done/STUMP CORRECTION	PUS	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	R	R	R	S	S		E	
89	50Y/M	248/60857	SGE-II-ISCU	ACUTE MESENTRIC ISCHEMIA/SEPSIS/	PUS	Klebsiella	pneumoniae subsp pneumoniae	R	R	R	S	R	S	S	R	R	P	M+K	
90	50Y/M	243/51348	SGE-III	Periampullarey CA.of pancreas/whipple's proceduredone	BILE	Klebsiella	oxytoca	R	R	R	S	R	S	S	S	R		M	
91	49Y/M	47/51456	SUR-III	SCROTAL WALL ABSCESS	PUS	Klebsiella	oxytoca	R	R	R	R	R	S	S	S	S		E	
92	40/F	49/58124	MED-I	DM/CVA/LT.HEMIPLEGIA/BED SORE	PUS	Klebsiella	oxytoca	R	R	R	R	R	S	S	S	S		E	
93	60Y/M	131/61017	MED-I	SHT/RECCURENT MI/URI	SPUTUM	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	S	S	S	S	S		E	
94	50Y/M	N10NV/57641	NEUROSUR-ISCU	ACCIDENTAL FALL/CVA/SDH	treacheal aspirate	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	R	R	R	S	S		E	
95	35Y/F	131/59966	MED-I	AFI/LRTI	SPUTUM	Klebsiella	oxytoca	R	R	R	S	S	S	S	R	R		M	
96	40Y/F	URO OP-III/94861	URO	RT RENAL CALCULUS/RECURRENT UTI	URINE	Klebsiella	pneumoniae subsp pneumoniae	R	R	R	S	R	S	S	S	S		E	
97	60Y/M	URO OP-III/94855	URO	BPH/CAUTI	URINE	Klebsiella	oxytoca	R	R	R	S	R	S	S	S	S		E+A	
98	45Y/F	212/61974	MED-III-IMCU	POST INGUINAL HERNIA REPAIR/ACUTE CKD/CAD/SEPSIS	PUS	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	R	R	R	R	R		R	
99	38Y/F	25/19049	ENT-4	CSOM RT EAR/POST OPDISCHARGE	PUS	Klebsiella	oxytoca	R	R	R	S	R	S	S	S	S		E	
100	49Y/M	241/38425	GICU	DM/CKD/BED SORE	PUS	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	S	S	S	S	S		E	
101	49Y/M	236/55659	PW/VS-I	LT RAW AREA/GRAFT DONE	PUS	Klebsiella	oxytoca	S	S	S	S	R	S	S	S	S		A	
102	48Y/F	9'S/57895	SUR-6	INFECTED PSEUDO CYST PANCREAS/ENDOSCOPIC ASPIRATION	PUS	Klebsiella	oxytoca	R	R	R	S	R	S	S	S	S		E+A	
103	20Y/F	263/57271	RTU-ISCU	IDIOPATHIC CKD/CADAVERIC TRANSPLANT	DRAIN TUBE	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	S	S	S	S	S		E	
104	56Y/M	29/65652	ORTHO-II	GRIII B COMP # BB LT LEG/ORIF	PUS	Klebsiella	oxytoca	R	R	R	S	S	R	R	R	R		M	

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105	42Y/M	OP/59020	TM-II	CHR.BRONCHITIS	Bronchial wash.	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	S	S	S	S	S		E	
106	39Y/M	54/56211	TM-II	COPD/Acute exaberation	Bronchial wash.	Klebsiella	pneumoniae subsp pneumoniae	R	R	R	S	S	S	S	S	S		E	
107	60Y/M	131/61400	MED-II	SHT/LRTI/COPD/ACUTE EXABERATION	SPUTUM	Klebsiella	oxytoca	R	R	R	R	R	S	S	S	S		E	
108	20Y/F	245/52316	SGE	ACUTE PANCREATIC ABSCESS/ENDOSCOPIC ASPIRATION	PUS	Klebsiella	oxytoca	R	R	R	R	R	S	S	S	S		E	
109	58Y/M	223/62690	GM-ICU	DM/CKD/CELLULTIS BOTH LL.	TISSUE	Klebsiella	oxytoca	R	R	R	R	R	R	R	R	R		R	
110	50Y/F	N5N6/43285	NEUROSUR-ISCU	ACUTE FLACCID PARALYSIS/SEMICONSCIOUS STATE	FOLEY'S CATHETER TIP	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	S	R	R	S	S		E	
111	60Y/M	CTPO/60183	CAR-ICU	POST CABG/STERNAL GRANULOMA	SINUS TRACT PUS	Klebsiella	oxytoca	R	R	R	S	R	S	S	S	S		E	
112	40Y/F	232/59207	SUR-3	DEGLOVING INJURY RT THIGH	PUS	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	S	S	S	R	R	P	K	PRESENT
113	50Y/M	125/63628	DM-2	DM/DFS/NON HEALING ULCER RT FOOT	PUS	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	S	S	S	S		E	
114	19Y/F	205/63378	ORTHO-II	RTA/CRUSH INJURY RT LEG PUO/SEPSIS	URINE	Klebsiella	oxytoca	R	R	R	S	R	R	R	R	R	P	M	
115	68Y/M	OP/69020	URO-III	BPH/UTI	URINE	Klebsiella	oxytoca	R	R	R	R	S	R	R	R	R		R	
116	60Y/F	9'S/63063	DM-I	Type 1 DM/IDDM?ulcer RT footURO SEPSIS	URINE	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	S	S	S	S	P	E+A	
117	20Y/M	29/49669	SUR	ACCIDENTAL FALL/DEEP INJURY LT LEG	PUS	Klebsiella	oxytoca	R	R	R	R	R	S	S	S	S		E	
118	49Y/M	30'S/58706	DM-III	DFS/NON HEALING ULCER	PUS	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	S	S	S	S		E	
119	50Y/M	125/6043	MED-II	DFS RT LEG	PUS	Klebsiella	oxytoca	R	R	R	R	R	S	S	S	S		E	
120	40Y/M	29'S/38059	ORTHO-II	DISC PROLAPSET12-L1/POST LUMBAR STABILISATION	PUS	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	R	R	R	S	S	P	E+A	
121	50Y/M	30'S/59957	SUR-II	SHT/DM/POST PARLYTIC SEQULAE/BED SORE	PUS	Klebsiella	oxytoca	R	R	R	S	R	S	S	S	S		E	
122	60Y/F	253/50600	SGE-II	CA.STOMACH/CORRECTIVE SURGERY/SSI	PUS	Klebsiella	oxytoca	R	R	R	R	R	S	S	R	R	P	K	PRESENT
123	27Y/F	212/58013	IMCU	POST LSCS/DIC/SEPSIS	TREACHEAL ASPIRATE	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	R	R	R	R	R		R	
124	19Y/M	N4N5/61823	NEUROSUR-IV-ISCU	HEAD INJURY/EDH/EXCISION DONE	TREACHEAL ASPIRATE	Klebsiella	pneumoniae subsp aerogenes	S	S	S	R	R	S	S	R	S		A	
125	50Y/M	124/62642	MED-111	SHT/CHR.BRONCHITIS/ACUTE EXAERBATION	SPUTUM	Klebsiella	pneumoniae subsp aerogenes	S	S	S	R	R	S	S	S	S		A	

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126	50Y/M	212/54184	IMCU	AFI/ADD/SEVEREDEHYDRATION HYPOVOL SHOCK	TREACHEAL ASPIRATE	Klebsiella	oxytoca	R	R	R	S	S	R	R	R	R	P	E+M	
127	60Y/M	123/62507	MED-III	COPD/Acute exaberationUTI	URINE	Klebsiella	oxytoca	R	R	R	R	R	S	S	S	S		E	
128	50Y/M	241/67562	HEPATOLOGY	CHR LIVER DISEASE/CHR.ALCOHOLIC	ASCITIC FLUID	Klebsiella	oxytoca	R	R	R	S	S	S	S	R	R	P	K	PRESENT
129	60Y/M	OP/58946	URO-2	RENAL CALCULUS/BPH/UTI	URINE	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	S	S	S	S		E	
130	60Y/F	43/58950	URO	STRICTURE OF URETHRA/CA.CX/RECURRENT UTI	URINE	Klebsiella	oxytoca	R	R	R	S	S	R	R	S	S		E	
131	50Y/M	22/52195	ORTHO-II	POST LUMBAR STABILISATION	PUS	Klebsiella	oxytoca	S	S	S	R	R	S	S	S	S		A	
132	20Y/M	224/60641	SGE-I	INTESTINAL OBSTRUCTION/APPENDICULAR PERFORATION, POST	PUS	Klebsiella	oxytoca	S	S	S	R	R	S	S	S	S		A	
133	19Y/M	21/60530	ORTHO-11	ACCIDENTAL INJURY/COMP#PATELLA PT/INF PATELLA NAIL	PUS	Klebsiella	oxytoca	R	R	R	R	R	S	S	S	S		R	
134	55Y/M	234/50935	SGE-11-ISCU	Periampullarey CA.of pancreas/whipple's proceduredone	DRAIN	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	R	S	S	S	R	P	M	
135	40Y/M	49/54605	VS-1-ISCU	TIBIAL ARTERY STENOSIS/POST ARTERY REPLACEMENT	PUS	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	R	S	S	S	S		E	
136	50Y/M	205/64519	ORTHO-II	#BB LEG RT /INFECTED IMPLANT.	PUS	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	S	R	R	S	S		E	
137	50Y/M	22/56224	ORTHO-1-ISCU	RTA/POLY TRAUMA/HEAD INJURY	PUS	Klebsiella	oxytoca	R	R	R	S	S	S	S	S	S		E+A	
138	24Y/M	263/53952	URO/RTU	IDIOPATHIC CKD/CADAVERIC TRANSPLANT	DRAIN	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	S	S	S	R	P	E+M	
139	49Y/M	233/63424	DM-II	DFS LT/BK AMPUTATION	PUS	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	S	S	S	R	P	K	PRESENT
140	20Y/F	162/55887	MED-111	SLE/PAPULES &PUSTULES OVER DORSAL PART	PUS	Klebsiella	oxytoca	R	R	R	R	R	S	S	S	R	P	E+M	
141	60Y/F	N4N5/60056	NEURO-ICU	SHT/CVA/SEMICONSCIOUS STATE	CATHETER TIP	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	S	S	S	S		E+A	
142	39Y/F	500/67594	SGE-2-ISCU	POTT'S SPINE/GIT BLEEDING	CATHETER TIP	Klebsiella	oxytoca	R	R	R	R	R	R	R	S	R	P	M+K	
143	52Y/F	223/58107	DM-2	SHT/DFS/BELOW KNEE AMPUTATION DONE	WOUND SWAB	Klebsiella	oxytoca	R	R	R	R	R	S	S	S	S		E	
144	32Y/M	222/60713	SUR-11	UMBILICAL HERNIA/POST MESH REPAIR/SSI	PUS	Klebsiella	oxytoca	R	R	R	S	R	S	S	S	S		E	
145	19Y/M	29/64929	ORTHO-II	REY'S AMPUTATION RT LEG/	PUS	Klebsiella	oxytoca	R	R	R	S	S	S	S	S	S		E	
146	51Y/M	132/63880	MED-111	COPD/Acute exaberation	SPUTUM	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	S	R	R	S	S		E	

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147	59Y/M	116/27981	MED-II	OLD TB/CHR.BRONCHITIS	SPUTUM	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	R	S	S	S	S		E	
148	24Y/M	N2N4/56252	NEUROSUR-NICU	RTA/HEADINJURY/EDH LT SIDE.	TREACHEAL ASPIRATE.	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	R	S	S	S	S		E	
149	59Y/M	N2N4/56256	NEUROSUR	CVD/LT.HEMIPLEGIA/SEMICONSCIOUS	TREACHEAL ASPIRATE.	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	S	S	S	S		E	
150	52Y/M	125/64594	MED-111	COPD/Acute exaberationUTI	URINE	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	R	S	S	S	R	P	K	PRESENT
151	60Y/M	9'S /69021	DM-1	DFS RT LEG/DKA/UTI.	URINE	Klebsiella	pneumoniae subsp aerogenes	S	R	R	S	R	S	S	S	S		A	
152	59Y/M	47/60040	URO	BPH/TURP	URINE	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	S	R	R	S	S		E	
153	30Y/M	29/64518	ORTHO-111	RTA/#BBLT.POST ILIZAROV TECH	PUS	Klebsiella	oxytoca	S	R	R	S	R	S	S	S	S		A	
154	19Y/M	29/64531	ORTHO-111	RTA/#RT FIBULA/K'WIRE FIXATION	PUS	Klebsiella	pneumoniae subsp aerogenes	S	R	R	S	R	S	S	S	S		E+A	
155	87Y/M	111/72037	MED-VI	SHT/ACUTE CVD/SEPSIS	BLOOD	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	S	S	S	R	P	E+M	
156	48Y/F	245/65459	SGE-111	CA.RECTUM WITH PROLAPSE/RADIATION PRURITIS	WOUND SWAB	Klebsiella	oxytoca	R	R	R	R	R	S	S	S	S		E	
157	35Y/M	131/69150	MED-111	CHR.ALCOHOLIC/LIVER CIRRHOSIS	ASCITIC FLUID	Klebsiella	oxytoca	R	R	R	S	R	S	S	S	S	P	K	PRESENT
158	61Y/M	218/65714	DM-1	DM/RECURRENT UTI	URINE	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	R	R	S	S		E	
159	19Y/F	212/68914	IMCU	AFI/THROMBOCYTOPENIA/SEPSIS	TRACHEAL ASPIRATE	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	S	S	S	S		E	
160	19Y/M	131/52314	MED-111	AFI/PUO/ THROMBOCYTOPENIA/URI	URINE	Klebsiella	oxytoca	R	R	R	S	R	S	S	S	S		E	
161	58Y/M	OP/76412	URO-111	DM/LT.RENAL CALCULUS/UTI	URINE	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	S	S	S	S		E	
162	38Y/M	225/63452	SUR-11	SCROTALTRAUMA	PUS	Klebsiella	oxytoca	R	R	R	S	R	R	R	S	S		E	
163	19Y/F	141/67695	MED-111	AFI/URTI/UTI	URINE	Klebsiella	oxytoca	S	R	S	S	R	S	S	S	S	P	A+M	
164	60Y/M	241/61664	GERIATRIC MEDICINE	CHR.BRONCHITIS	SPUTUM	Klebsiella	oxytoca	R	R	R	S	R	S	S	S	S		E	
165	29Y/M	20-A/66441	ORTHO-11	RTA/GR-111B COMP #SHAFT OF HUMERUS/ORIF	PUS	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	S	S	S	S		E	
166	46Y/F	9'S/57787	SUR-VI	DFS LT FOOT	PUS	Klebsiella	oxytoca	R	R	R	S	S	S	S	S	S		E	
167	19Y/F	212/67131	IMCU	AFI/AKD/UTI	HIGH VAGINAL SWAB	Klebsiella	oxytoca	R	R	R	R	R	S	S	S	R	P	M	

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168	20Y/F	164/18796	ENT-111	CSOM LT EAR/POST MRM	AURAL SWAB	Klebsiella	oxytoca	R	R	R	R	R	R	R	R	R	P	M	
169	76Y/M	OP/27981	TM-11	COPD/Acute exaberation/UTI	URINE	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	S	S	S	S		E	
170	19Y/F	245/49786	SGE-11-ISCU	PSEUDO CYST OF PANCREAS/ENDOSCOPIC ASPIRATION	PUS	Klebsiella	oxytoca	R	R	R	S	R	S	S	S	S		E	
171	30Y/M	29/60525	URO/RTU	IDIOPATHIC CKD/CADAVERIC TRANSPLANT	FOLEY'S TIP	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	S	S	S	S		R	
172	60Y/M	167/61409	ORTHO-111	GR 11B COMP #BBLT LEG/ORIF	PUS	Klebsiella	pneumoniae subsp aerogenes	S	S	S	R	R	S	S	S	S		A	
173	60Y/M	43/60631	VS-11-ISCU	CVD/MI/RT TRANSFEMORAL EMBOLECTOMY DONE	CLOT	Klebsiella	oxytoca	R	R	R	S	R	S	S	S	S		E	
174	45Y/F	500/62279	PW/SUR-I	CELLULITIS RT LEG	PUS	Klebsiella	pneumoniae subsp aerogenes	S	R	R	S	S	R	R	S	S		A	
175	60Y/M	47/63756	URO-11	BPH/CKD/UTI	URINE	Klebsiella	oxytoca	R	R	R	R	R	S	S	S	S		E	
176	29Y/M	213/63559	SICU	RTA/GAS GANGRENE RT THIGH	PUS	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	R	S	S	S	S		E	
177	60Y/F	254/29141	ORTHO-1V	TKR/ASPIRATION PNEUMONIA	TRACHEAL ASPIRATE	Klebsiella	oxytoca	R	R	R	R	R	S	S	S	S		E	
178	20Y/M	47/29154	URO-1	RECURRENT UTI/IDIOPATHIC CKD	URINE	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	R	S	S	S		E	
179	29Y/M	49/29168	URO-1	RT RENAL CALCULUS/RECURRENT UTI	URINE	Klebsiella	oxytoca	S	R	R	R	R	S	S	S	S		A	
180	20Y/M	30'S/25809	SUR-111-ISCU	ACCIDENTAL INJURY LT PELVIS/HEMATURIA	URINE	Klebsiella	oxytoca	R	R	R	R	R	S	S	S	S		E	
181	20Y/F	162/28924	RHEUMATOLOGY	SLE/LYMPHADENOPATHY/LUPUS NEPHRITIS	URINE	Klebsiella	oxytoca	R	R	R	R	R	S	S	S	R	P	E+M	
182	20Y/M	49/68043	ORTHO-11	RTA/CRUSH INJURY RT LEG	PUS	Klebsiella	pneumoniae subsp aerogenes	S	S	S	R	R	S	S	S	S		E+A	
183	19Y/M	32/19562	ENT-11	LT CSOM/MRM	PUS	Klebsiella	oxytoca	S	R	S	S	R	S	S	S	S		E+A	
184	29Y/M	25/69240	SUR-1V-ISCU	DUODENAL PERFORATION/POST LAPROTOMY	PUS	Klebsiella	oxytoca	S	S	S	S	R	R	R	R	R		R	
185	18Y/M	213/69145	SGE-111	INFECTED PSEUDO CYST PANCREAS	DRAIN FLUID	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	R	S	S	S	S		M	
186	75Y/M	49/68370	VS-11-ISCU	DM/DFS/LT AK AMP/INFECTED GRAFT TISSUE	TISSUE	Klebsiella	oxytoca	R	R	R	S	S	S	S	S	S		E	
187	18Y/F	N4N5/60880	NEUROSUR	Post meningitis hydrocephalus/RT VP shunt.	CSF	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	S	S	S	S		E	
188	20Y/M	46/68968	NEPHRO-ISCU	IDIOPATHIC CKD/CADAVERIC TRANSPLANT	PERITONEAL FLUID	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	S	S	S	S		E	

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189	60Y/M	32/67836	TM-11	CHR.BRONCHITIS/CHR SMOKER/ACUTE EXAERBATION	Bronchial wash.	Klebsiella	oxytoca	R	R	R	R	R	S	S	S	R	P	M	
190	55Y/M	OP/35802	URO-111	TYPE 2 DM/RECURRENT UTI	URINE	Klebsiella	oxytoca	S	S	S	S	S	R	R	S	S		E	
191	20Y/F	N3N4/31214	NS-11-NICU	HEAD INJURY/EDH/EXCISION DONE	TRACHEAL ASPIRATE	Klebsiella	oxytoca	R	R	R	R	R	S	S	R	R		R	
192	52Y/M	49/66821	NEPHRO	SHT/CKD/ON DIALYSIS	PERITONEAL FLUID	Klebsiella	oxytoca	R	R	R	S	S	S	S	S	S		E	
193	55Y/M	OP/63630	TM-11	OLD TB/CHR.BRONCHITIS	Bronchial wash.	Klebsiella	pneumoniae subsp aerogenes	R	R	R	R	R	S	R	R	R		R	
194	20Y/M	43/90072	URO-111	ACCIDENTAL INJURY/INFL PERITONITIS	PERITONEAL FLUID	Klebsiella	oxytoca	R	R	R	R	R	R	R	R	R	P	M+K	
195	60Y/F	143/59427	MED-111	SHT/GIDDINESS/LRTI	THROAT SWAB	Klebsiella	oxytoca	R	R	R	R	R	S	R	R	R		R	
196	40Y/F	42/59949	URO-11	RENAL CALCULUS/UTI	URINE	Klebsiella	oxytoca	R	R	R	S	S	S	R	R	S	P	E+M	
197	60Y/M	201/59717	NEPHRO	URETHERAL STRICTURE/RECURRENT UTI	DRAIN	Klebsiella	pneumoniae subsp aerogenes	S	S	S	R	R	S	R	R	R		R	
198	40Y/M	29/65302	HEPATOLOGY	CIRRHOSIS/ALCOHOLIC HEPATITIS	ASCITIC FLUID	Klebsiella	oxytoca	R	R	R	S	S	S	S	S	S		E	
199	60Y/M	157/35525	RADIOTHERAPY	CA.GLOTTIS ON CHEMO	TRACHEAL ASPIRATE.	Klebsiella	pneumoniae subsp aerogenes	R	R	R	S	R	S	S	S	S		E	
200	19Y/F	212/66139	IMCU	GBS/ASC PARALYSIS LL	CATHETER TIP	Klebsiella	pneumoniae subsp aerogenes	S	S	S	S	R	R	R	S	S		A	